Blood Diseases of
Infancy and Childhood

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CARL H SMITH M A M D

Professor of Clinical Pediatrics Cornell University Medical College New York, N Y Attending Pediatrician The New York Hospital New York N Y Consulting Pediatrician Beekman Downtown Hospital New York N Y Fitkin Memorial Hospital Neptune N J Misericordia Hospital New York N Y New York Infirmary New York, N Y St Joseph s Hospital Far Rockaway N Y Sea View Hospital Staten Island N Y Consulting Hematologist in Pediatrics Lenox Hill Hospital New York N Y

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To my wife
MARGARET

Preface

The purpose of this book is to present the essentials of pediatric hematology in concise form for the medical student and practitioner. In lectures over a period of many years to members of these groups I have been impressed with the need for providing a textbook in which the salient features of blood dyscrasias are presented against the background of normal development of infancy and childhood.

Although these hematologic disorders correspond closely to those occurring in adult life, the approach to diagnosis is frequently complicated by concomitant alterations which normally take place during growth within and outside of the hematopoietic system. Certain of the blood disorders occur almost exclusively in early life and are best described in a pediatric setting.

The rapid development of pediatric hematology can be strikingly illustrated by recalling the fate of von Jaksch's anemia. This syndrome which dominated the discussions of anemia in pediatric textbooks thirty years ago receives scant mention or is entirely omitted from present day publications. The dismemberment of this heterogeneous group of blood disorders replaced by more soundly based conditions reflects the evolution of new concepts in a rapidly expanding field of medicine. Similarly, such diverse conditions as thrombocytopenic purpura, erythroblastosis, and the hemolytic anemias and disorders of the coagulation mechanism, which occur so prominently in younger persons, have been clarified by recent contributions from the growing field of immunohematology and by the identification of multiple factors responsible for blood clotting. In the hereditary hemolytic syndromes important information has been acquired by the application of genetic analysis and the characterization of abnormal types of hemoglobin by electrophoretic examination. The discovery that drug sensitivity and favism are based on a genetically transmitted enzyme deficiency suggests that other unexplained hematologic entities may also eventually be included in the growing list of inborn errors of metabolism.

The search for a fetal etiology of congenital anomalies has also had its impact on the blood dyscrasias in the pediatric age group. This orientation has been so attractive that the pathogenesis and interpretation of the blood disorders especially in the early months and years of life require an examination of the maternal-fetal relationships and of environmental and genetic influences and a consideration of normal embryologic development and its aberrations.

Although the blood diseases of younger patients are effectively presented in the comprehensive hematologic textbooks currently available nevertheless there is much to be gained by their separate consideration This book is not intended however to supplant these larger works rather it is designed to serve as a companion volume since few diseases in this or other specialties can be arbitrarily restricted to a specific age period It is planned throughout to emphasize the pathogenesis of hematologic disorders of the pediatric age group in the light of established concepts as a basis for rational treatment

From the rapidly enlarging mass of hematologic and pediatric contributions only that segment is represented which bears pertinently on the interpretation diagnosis and management of the blood diseases encountered in pediatric practice

Elaborate techniques have been developed within recent years permitting visualization of physiologic forces controlling blood formation and their abnormalities Although certain important data can be acquired with these facilities I have always felt that the detection of the common blood disorders lies within the province of every medical practitioner by careful history and physical examination with the use of simple instruments and techniques and by coordinating information derived from a variety of interrelated sources In striving to achieve this objective, it is hoped that an easily understandable and practical book has been prepared

I wish to acknowledge my indebtedness to the authors of the standard hematologic pathologic and pediatric textbooks which were of inestimable value for orientation and subject matter Thanks are due to my colleagues and associates who offered suggestions in various aspects of the text many of whom are identified in papers written conjointly and are mentioned in the bibliographies I wish particularly to express my appreciation to those who reviewed one or more chapters of the book and whose advice and criticism were invaluable notably Dr Marion E Erlandson Dr Sydney S Gellis Miss Jane Haber Dr S Frederick Rabiner and Dr Marjorie B Zucker Above all I am everlastingly indebted to my wife Margaret for supervising and contributing to every phase of the preparation of the manuscript thereby relieving me of numerous tasks that disturb the equanimity needed for writing For added participation of my family thanks are due my daughter Christine for help in revising the manuscript and my son Carl for emphasizing the point of view and needs of the medical student My deep appreciation is extended to Dr Samuel Z Levine Professor of Pediatrics of the Cornell University Medical College who by his constant support and warm friendship provided me with the needed encouragement and stimulation to develop the field of pediatric hematology in his department and the material on which this book is based Thanks are due Mr Percy Brooks and his associates of the Department of Illustration of The New York Hospital Cornell Medical Center for cooperation in the preparation of the figures Sincere appreciation is due Mrs Pandora Manning for her patience in typing and retyping large sections of the manuscript

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CHAPTER 1

Origin and Development of Blood Cells

Blood Formation in the Fetus A review of the essential features of prenatal blood development provides a basis for interpreting postnatal abnormalities of the circulating blood elements their progenitors and sites of formation The designation of prenatal hematopoiesis imparts an implication of continuity to the events occurring in the embryo in the first two months and in the fetus in the remainder of gestation Current emphasis on maternal fetal relationships which has shed so much light on other systems conceivably may clarify the etiology of certain of the blood dyscrasias on the same basis An understanding of the blood changes in the earlier months of life furthermore requires some knowledge of embryonic and fetal blood formation

Sites of Blood Formation Blood cells in the embryo arise from the mesenchyme The first cells produced in the yolk sac become the primitive red corpuscles Since the mesenchyme is widespread throughout the embryo blood formation begins in multiple sites but eventually becomes specialized in certain organs With some overlapping definitive blood centers mainly involving red cell elements appear successively in the yolk sac at the fourth month of gestation [3] Although the bone marrow makes its appearance in the sixth week of development it does not become a site of active hematopoiesis until the fourth to fifth month and does not become the exclusive site[3] until two to three weeks after birth [1] Until the middle of fetal life the liver is the most actively engaged organ participating in blood formation It has been stated that the liver as opposed to the marrow represents the principal source of fetal erythrocytes In this period of hepatic hematopoiesis fetal hemoglobin is the sole type synthesized As hematopoiesis wanes in this area it is assumed by the bone marrow which exercises this function for the remainder of fetal life Coincidental activity goes on in the spleen lymph nodes and to a lesser extent in the thymus

The bone marrow and spleen provide an ideal environment for red cell and hemoglobin formation In both there are nonanastomosing arterial capillaries emptying into a rich plexus of venous sinusoids By virtue of sluggish circulation and blood stasis a relatively high carbon dioxide tension develops a factor of

lation possesses completely differentiated precursors Myeloblasts are precursors of myelocytes and granulocytes lymphoblasts of lymphocytes and monoblasts of monocytes Reticulocytes and mature erythrocytes are derived from pronormoblasts and the more mature normoblasts Erythroblasts refer either to megaloblasts or pronormoblasts The former occur in patients with pernicious anemia The latter are the progenitors of the normal red cell series The megakaryoblast and promegakaryocyte are the primitive forms of the mature megakaryocyte

Fetal Hemoglobin The earliest evidence of a difference between fetal and maternal hemoglobin was the greater resistance to alkaline denaturation observed in fetal hemoglobin Fetal hemoglobin constitutes about 45 to 90 per cent of the hemoglobin of the infant at birth and is rapidly replaced within the first year by adult hemoglobin Normally about 15 per cent fetal hemoglobin persists to the age of 1 year 5 per cent to 2 years and less than 2 per cent after the age of 4 years[6] It is rarely demonstrable thirty months after birth Occasionally a child continues to have small quantities of alkali resistant hemoglobin as late as 4 years of age[6]

Cook and co workers have shown that in infants born after more than 34 weeks gestation there is an inverse relation between gestational age and percentage of fetal hemoglobin Most infants born after less than 36 weeks gestation had more than 90 per cent fetal hemoglobin at birth and those born after 36 weeks usually had less than 90 per cent at birth After 34 weeks gestation the percentage of fetal hemoglobin drops approximately to 3 or 4 per cent per week prenatally which is similar to the postnatal weekly decrease

Fetal Hemoglobin Content as an Index of Maturity The content of fetal hemoglobin has been advanced as an approximate index of maturity especially in the overmature newborn infant Cottom[8] observed a close correlation between the percentage of fetal hemoglobin and the period of gestation but not with the birth weight The mean fetal hemoglobin ranged from 90 per cent at 35 weeks gestation to values below 60 per cent at 42 weeks A similar observation was made by Abrahamov and associates[1] who found an average fetal hemoglobin concentration of 85 5 per cent (range 64 1 to 95 per cent) in the normal control group as compared with an average of 65 1 per cent in the postmature newborn infant (range 54 4 to 76 4 per cent)

Fetal Blood and Oxygen Dissociation Fetal blood takes up oxygen at a tension at which it is relinquished by maternal blood a mechanism of distinct advantage to the fetus Eastman and associates[11] found that at gas tensions between 25 and 60 mm Hg *fetal blood absorbs oxygen more effectively and that at all* gas tensions it releases carbon dioxide more readily than does the blood of the mother The dissociation curve of fetal blood thus is displaced to the left as compared with that of the pregnant and nonpregnant woman a circumstance favorable to the uptake of oxygen by the fetus in utero[15] Barcroft[4 5] suggested that the greater affinity for oxygen of fetal blood than maternal blood depends upon the fetal type of hemoglobin a type which was thought to acquire oxygen readily and shed it with difficulty

Although it is true that the fetal type of hemoglobin differs chemically from normal adult hemoglobin (see discussion of fetal hemoglobin) evidence that it

primary importance in the elaboration of hemoglobin and in the formation of the primordial cell

Appearance of Blood Elements The mesenchyme is regarded as the essential blood forming tissue of the embryo corresponding to fixed connective tissue cells in the adult organism The hemocytoblast a derivative of the mesenchyme is the primitive totipotent cell whose main function is involved in hematopoiesis This early cell frequently ameboid in embryos represents the precursor of red blood cells granular leukocytes lymphocytes and megakaryocytes [10,1]

ERYTHROCYTES In each area of blood formation the erythroblasts multiply rapidly by mitosis and as they mature manifest in sequence the generation of hemoglobin condensation and subsequent loss of the nucleus and final entrance into the circulation In the first six weeks of gestation practically all red cells are nucleated A rapid change to non nucleated forms occurs during the ninth week and is practically complete by the end of the tenth week

The very large primitive erythroblasts representing the first or provisional generation of red cells to appear in the embryo are replaced by the smaller and more numerous erythroblasts formed in the hepatic period By the end of the fourth month the primitive cells have entirely disappeared

GRANULOCYTES AND LYMPHOCYTES Granulocytes noted initially in the yolk sac and liver are found in increasing numbers in the fourth month In the spleen red cell formation is extremely active initially but by the fifth month of gestation it gives way to the formation of lymphocytes [8] In another study[15] it was found that red cells alone were in evidence in the blood for the first two and one half months of intrauterine life At the end of this time granulocytes made their appearance with the lymphocytes appearing at the beginning of the fourth month

MONOCYTES Although it is still debatable the origin of monocytes has been relegated to the reticular cells of the reticuloendothelial tissue From these cells in the liver lymph nodes spleen and marrow the progenitor of the monocytes the monoblast is derived

PLATELETS Megakaryocytes are noted in the yolk sac and liver however with intensive production of erythroblasts in the liver granulocytes and to a lesser extent megakaryocytes are formed The latter increase in number as the hepatic period of blood formation becomes more vigorous but platelet proliferation from megakaryocytes is not active until the bone marrow period Although platelets appear in the embryo at the same time as megakaryocytes and are derived from them the origin of the megakaryocytes has been variously traced to hemocytoblasts reticuloendothelium or fused histiocytic elements In extrauterine life evidence has been cited of active platelet formation from megakaryocytes in the lungs as well as the bone marrow [14]

Theories of Blood Formation There are two theories as to the origin of blood formation The monophyletic theory regards the hemocytoblast as the totipotential cell from which all types of blood elements both red and white originate According to the polyphyletic theory each of the blood cell elements in the peripheral circulation possesses completely differentiated precursors According to the polyphyletic theory each of the blood cell elements in the peripheral circu

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Although it is true that the fetal type of hemoglobin differs chemically from normal adult hemoglobin (see discussion of fetal hemoglobin) evidence that it

is responsible for the fetal type of oxygen dissociation is inconclusive Prepared hemoglobin solutions from fetal and maternal blood possess similar affinities for oxygen It has not yet been determined whether fetal hemoglobin residing within the intact erythrocytes absorbs oxygen at low concentrations more effectively than normal blood The properties ascribed to fetal blood possibly may depend upon factors inherent in the intact red cell in which fetal hemoglobin plays an influential part

On the other hand the differences in oxygen uptake in the fetal as compared with the adult red cells may be due to properties in the red cells or plasma exclusive of fetal hemoglobin It is also conceivable that differences in thickness or thinness of the maternal and fetal red cells may account for the peculiarities of oxygen dissociation and uptake in fetal life On this basis the maternal red cells which are thinner than those of the fetus [6] would promote oxygen transfer to the fetus [3] where it would be retained because of the greater red cell thickness

Relation of Cord Blood Hemoglobin to Fetal Oxygen Saturation The mean umbilical cord hemoglobin obtained by most observers ranges between 15 7 and 17 9 gm per 100 ml of blood [17] The wide spread of hemoglobin values observed by Marks and co workers [1] extending from 12 to 22 gm per 100 ml of blood (95 per cent range of 13 7 to 20 1 gm) with a mean value of 16 9 gm is representative of values noted in most series Marks and colleagues[17] also showed that whether an infant is born with a cord hemoglobin as low as 13 gm or as high as 20 gm the hemoglobin will be established at a level of about 11 gm at 2 months of age

Studies of the cord blood from infants of varying age of fetal maturity have been analyzed from the standpoint of influences acting on the fetus before birth Several factors particularly have been investigated the state of oxygen saturation the hemoglobin concentration and a possible relationship between the two Walker and Turnbull [4] found that the hemoglobin level during gestation rose steadily from 9 gm per 100 ml in the tenth week to 14 or 15 gm by the twenty second to the twenty fourth week By the thirty eighth week the mean value was 15 2 gm and by the fortieth week it was 16 5 gm When pregnancy was prolonged this rise continued By the forty third week the mean value was 18 8 gm They interpreted the increasing hemoglobin value as a response to a falling oxygen supply in the fetus and furthermore decided that postmaturity was associated with abnormally high hemoglobin levels

Marks and associates[1] and Rooth and Sjostedt [5] could find no correlation between cord blood hemoglobin and fetal gestation time The latter were unable to confirm Walker and Turnbull s [4] findings that there is a continuous decrease in oxygen saturation with advancing pregnancy They found a constant mean hemoglobin level of 17 gm per 100 ml from the fortieth to the forty third week and during these four weeks there was no correlation between cord blood hemoglobin and the duration of pregnancy On the other hand McKay [16] observed the gradual *reduction in the oxygen levels* of the cord blood in normal pregnancy as term is approached and passed but no relation to hemoglobin levels

Cook and co workers[7] also showed that intrauterine hypoxia in the postmature

infant was not associated with an increase in percentage of fetal hemoglobin On the basis of reticulocyte counts and clinical examination they suggested that increased total concentrations of hemoglobin observed in the postmature infant with prenatal hypoxia are possibly the result of hemoconcentration rather than an erythropoietic response to lack of oxygen

Despite these conflicting observations it may be stated that oxygen saturation *in the fetus is decreased but that additional data are required with techniques* designed to obtain precise intrauterine fetal measurements At present additional causes for the variation in cord hemoglobin levels at birth other than the factor of decreased oxygen saturation alone must be sought

Survival Time of Fetal Red Cells It is of interest that the life span of fetal red cells when measured by tagging umbilical cord blood at birth with radioactive chromium[13] is shorter than that of normal adults Recent studies indicate however that this difference in survival rates of the adult and of the newborn red cells as determined by Cr^{51} is only apparent since elution of the isotope from cord blood hemoglobin after dialysis is considerably greater than that from adult hemoglobin The method of a differential agglutination also demonstrates that the survival of red cells from infants is only slightly less than that from adults[10] By using Cr^{51} tagged erythrocytes and autotransfusion or heterotransfusion methods red cell survival of premature and full term infants was also found at normal adult levels during the first week of life but was reduced at the second and third months[11]

Fetal Myoglobin Human myoglobin may also be separated into fetal and adult types Fetal human myoglobin (MbF) exhibits a distinct spectroscopic and electrophoretic behavior which is different from adult muscle pigment (MbA) The muscles of premature and normal newborn infants contain only MbF which is gradually replaced by MbA within the first six months of life comparable in sequence to the period of disappearance of fetal red cell hemoglobin from the blood stream[1] The purpose and function of both the fetal hemoglobins and myoglobins have not been definitely established

Bone Marrow at Birth Infancy and Childhood At birth the bone marrow assumes the role of the dominant hematopoietic organ but the potencies of mesenchymal cells or of their reticular derivatives in the connective tissues for blood formation persist throughout life In older infants and children in whom the usual blood sites are strained as in those with the hereditary hemolytic diseases and leukemia compensatory hematopoiesis occurs quite readily in the liver and in the spleen

So active is the demand for blood cell formation that in the infant and young child all the bones are filled with red marrow Fat appears in the long bones at about 5 to 7 years of age although incipient changes may occur during infancy From that time until about the age of 20 years a gradual retrogression occurs so that active marrow is eventually restricted to the trunk (ribs sternum and vertebrae) and proximal portions of the femur and humerus Red marrow is also found in the clavicles scapulas skull and pelvis[9] With the appearance of nonfunctioning yellow marrow a potential reservoir is created for active blood formation in periods of stress

REFERENCES

1 Abrahamov A Salzberger M and Bromberg M Fetal Hemoglobin in Postmature Newborn Infants Am J Clin Path 26 146 1956
2 Allen D W Wyman J Jr and Smith C A The Oxygen Equilibrium of Fetal and Adult Human Hemoglobin J Biol Chem 203 81 1953
3 Arey L B Developmental Anatomy—A Textbook and Laboratory Manual of Embryology ed 6 Philadelphia 1954 W B Saunders Co
4 Barcroft J Conditions of Fetal Respiration Lancet 2 1021 1933
5 Barcroft J Fetal Circulation and Respiration Physiol Rev 16 103 1936
6 Chernoff A I and Singer K Studies on Abnormal Hemoglobins Persistence of Fetal Hemoglobin in Erythrocytes of Normal Children Pediatrics 9 469 1952
7 Cook C D Brodie H R and Allen D W Measurement of Fetal Hemoglobin in Newborn Infants Pediatrics 20 272 1957
8 Cottom D G Foetal Haemoglobin and Postmaturity J Obst & Gynaec Brit Emp 62 945 1955
9 Custer R P An Atlas of the Blood and Bone Marrow Philadelphia 1949 W B Saunders Co
10 Downey H Handbook of Hematology New York 1938 Paul B Hoeber Inc
11 Eastman N J Geiling E M K and DeLawder A M Foetal Blood Studies Oxygen and Carbon Dioxide Dissociation Curves of Foetal Blood Bull Johns Hopkins Hosp 53 246 1933
12 Gilmour J R Normal Hemopoiesis in Intrauterine and Neonatal Life J Path & Bact 52 25 1941
13 Hollingsworth J W Life Span of Fetal Erythrocytes J Lab & Clin Med 45 469 1955
14 Howell W H and Donahue D D The Production of Blood Platelets in the Lungs J Exper Med 65 177 1937
14a Kaplan E Studies of Red Cell Survival in Early Infancy A M A J Dis Child 98 603 1959
15 Knoll W Untersuchungen über embryonale Blutbildung beim Menschen Ztschr mikr Anat 18 199 1929
16 MacKay R B Observations on the Oxygenation of the Foetus in Normal and Abnormal Pregnancy J Obst & Gynaec Brit Emp 64 185 1957
17 Marks J Gairdner D and Roscoe J D Blood Formation in Infancy Part III Cord Blood Arch Dis Childhood 30 117 1955
18 McCarthy E F Oxygen Affinity of Human Maternal and Foetal Hemoglobin J Physiol 102 55 1943
19 Mollison P L Blood Transfusion in Clinical Medicine Springfield Ill 1956 Charles C Thomas Publisher
20 Rooth G and Sjostedt S Haemoglobin in Cord Blood in Normal and Prolonged Pregnancy Arch Dis Childhood 32 91 1957
21 Singer K Angelopoulo B and Ramot B Studies on Human Myoglobin II Fetal Myoglobin Its Identification and Its Replacement by Adult Myoglobin During Infancy Blood 10 987 1955
22 Suderman H J White F D and Israels L G Elution of Chromium 51 From Labeled Hemoglobins of Human Adult and Cord Blood Science 126:650 1957
23 Valtis D J and Baikie A G The Influence of Red Cell Thickness on the Oxygen Dissociation Curve of Blood Brit J Haemat 1 152 1955
24 Walker J and Turnbull E P N Haemoglobin and Red Cells in the Human Foetus Lancet 2 312 1953
25 Windle W F Physiology of the Fetus Philadelphia 1940 W B Saunders Co
26 Wintrobe M M Clinical Hematology Philadelphia 1956 Lea & Febiger

CHAPTER 2

Blood Changes During Growth—Postnatal Period, Infancy, and Childhood

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Knowledge of the normal blood values in the growing period is a prerequisite to the interpretation of a particular blood response in infancy and childhood For this reason the normal changes in hematologic values for each of the developmental periods are included in this discussion *It is recognized that the values to be cited are subject to wide variations both in each individual and among members of an age group These values are given as yardsticks based on experience and in terms of which abnormalities of graded severity may be judged and are in general agreement with the data available in standard texts* and articles dealing with this subject[7 9 10 ... 5 9]

Blood Changes in the Newborn Infant During the first week of life the decline in hemoglobin and red cells in the peripheral blood is at a minimum Following this initial stationary period a definite decline sets in The postnatal adjustment is characterized by a normal or slightly increased rate of blood destruction accompanied by diminished or stationary hematopoietic activity During this period the drop in red cells occurs within the limits of normal survival—namely 100 to 120 days or approximately 0 8 per cent per day The changes are usually orderly and gradual and appear to be directed toward the establishment of hematopoietic equilibrium designed to function at a lower level than exists at birth Although hemolysis accounts for the fall in hemoglobin and red cells the continued drop represents a diminished rate of compensatory regeneration The fall in hemoglobin represents a gradual adjustment to the increased oxygen saturation of the blood which prevails when the lung replaces the placenta as a source of oxygen Gairdner and co-workers[3] believe that erythrogenesis ceases when the oxygen saturation at birth is elevated from about 65 per cent in the umbilical vein to 95 per cent a few hours after birth Accordingly red cell formation is at a minimum until the hemoglobin level reaches 11 to 12 gm per 100 ml when regeneration sets in This regulation by the arterial oxyhemoglobin level implies that during the neonatal period decreases below 11 to 12 gm per 100 ml induce hematopoietic activity whereas elevations above 11 to 12 gm tend to decrease bone marrow activity Increased reticulocyte formation can be

observed in the anemic infant in the first days of life presumably from blood loss during delivery or the seepage of blood into the maternal circulation.

Physiologic Anemia of the Newborn Infant. Physiologic anemia of the newborn infant occurs in the first two or three months of life when the red cells and hemoglobin drop concurrently and in equal degrees. This decline proceeds in an orderly fashion and is not accelerated by hyperhemolysis unless pathologic factors set in. Following this neonatal adjustment, hemoglobin reaches its lowest level at 6 weeks to 2 months of age and remains fairly constant for the remainder of infancy. During the first two years of life the hemoglobin usually ranges from approximately 10 to 12 gm. per 100 ml. of blood.

Bone Marrow Changes. During the first week of life when the decline in hemoglobin and red cells is at a minimum, a marked drop in nucleated red cells occurs in the bone marrow. The lowest levels are reached between the end of the first week and the fourth week of life, with a considerable increase in cells following this period. For example, Shapiro and Bassen[10] found that on the first day of life the percentage of myeloid cells (neutrophils, myelocytes and myeloblasts) average 61 per cent and nucleated red cells 32 per cent. On the eighth day the myeloid percentage increases to 77 per cent, whereas the erythroid elements drop sharply to 12 per cent. At about 2 months of age, as the marrow erythroid activity returns, the peripheral blood shows an increase in reticulocytes and a rise in hemoglobin.

Blood Volume. The blood volume of the full term infant at birth is approximately 85 ml. per kilogram and that of the premature infant averages 108 ml., the difference being due mainly to an excess of plasma in the latter.[17] Normal blood volumes of 75 to 80 ml. per kilogram, which are the values in the adult, are reached after the second month. In the treatment of erythroblastosis the blood volume for both full term and premature infants is calculated on the basis of 85 ml. of blood per kilogram.

Hemoglobin Concentration. The cord blood hemoglobin varies approximately between 16.6[4] and 17.1 gm. per 100 ml. of blood.[7] In our laboratory the hemoglobin averaged 16.4 gm. per 100 ml., with a range from 14 to 19 gm. per 100 ml.

Gardner and associates compared the composition of cord blood at birth with venous blood and concluded that the elevation of the hemoglobin level during the first day of life is due to the shift of fluid (namely, whole plasma) from the circulation and is independent of placental blood transfer. The postnatal rise in hemoglobin and packed cell volume effected by the shift of fluid from the vascular compartment takes place within a few minutes after birth and is complete within an hour or two, reaching a value of 19.1 gm. per 100 ml. of hemoglobin (S.D. 2.36) in one to eight hours (as compared with 16.6 gm. in cord blood). The extent to which placental blood transfer contributes to this rise has been controversial.

According to Haselhorst and Allmeling, the average amount of blood in the placental vessels is 105 ml., with a range from 50 to 200 ml. Infants receive varying amounts of this blood depending upon the time after delivery at which the cord is clamped: 51 per cent of the blood reaches the infant in the first minutes after delivery, 79 per cent in five minutes and 91 per cent in the first ten minutes. It is usually estimated that the placenta contains a reservoir of 125 to 150 ml. of blood and that delayed clamping may account for the addition of as much as 100 ml. of blood to the circulation of the newborn infant. It is claimed that the increased red cell and hemoglobin mass resulting from late clamping becomes apparent after birth only as the plasma volume is readjusted and equalized. DeMarsh and associates observed

that hematocrit values were higher for the infants whose placental blood had been allowed to pass into their circulation at birth, averaging 61 per cent on the first day and 60 per cent on the third day. In those infants who failed to receive placental blood, the hematocrit values averaged 50 per cent on the first day and 51 per cent on the third day, practically the same as the average hematocrit reading of the umbilical cord blood, which was 51 per cent. It seems most likely that the placental transfusion of substantial amounts of blood by late clamping of the cord should be reflected in an elevation in hemoglobin soon after birth. In another study[*] no significant difference was found in erythrocyte values in the neonatal period with respect to the time of clamping unless the cord was deliberately stripped.

Blood samples from heel puncture show a higher concentration of hemoglobin than those from the vein. This may be largely due to the stasis in the peripheral blood of newborn macrocytic red cells. Mollison and Cutbush[10] give the following comparative normal hemoglobin values in newborn infants: with cord blood hemoglobin of 13.6 to 19.6 gm. per 100 ml., the venous blood on the first day increases to 14.5 to 22.5 gm., with corresponding skin prick values of 15.4 to 22.8 gm. These variations are important in the management of erythroblastosis (see Chapter 10).

The drop in hemoglobin to 11 gm. per 100 ml. of blood in the first two years of life is followed by a gradual rise, reaching a maximum at 14 years of 16 gm. for males and 14 gm. for females, with an average of 15 gm. for both sexes.

Erythrocyte Count. The red cell count is high at birth and averages 5.5 million, with a range from 5 to 6 million per cubic millimeter. The red cells are macrocytic at birth. They range in size from 8 to 9 microns in diameter, decrease to their smallest size of about 5 microns after three to six months, and rise to adult dimensions of 7.2 to 7.5 microns at eight months.

The red blood cells average 4.6 million at the end of the first year and 4.8 million at the end of twelve years. At 14 years of age and over males average 5.4 million red cells per cubic millimeter and females average 4.8 million.

Abnormally high erythrocyte values of unknown etiology were reported at birth in an infant with anorexia, lethargy, cyanosis, and convulsion.[*] The clinical signs subsided when blood was withdrawn and replaced with plasma. No congenital abnormalities were found. The etiology of this syndrome is unknown. In another case polycythemia in one twin and anemia in the other occurred in single-ovum twins with hemoglobin values of 25.2 and 3.7 gm. and red cell counts of 7.47 and 1.85 million per cubic millimeter, respectively. This discrepancy was explained on the basis of an arteriovenous shunt between the supposedly separate placental circulations which resulted in a parabiotic circulatory system. Unequal functioning between the two circulations accounted for the sharp differences in blood levels. In this case withdrawal of blood from the polycythemic infant was followed by an uneventful course. Usually, newborn polycythemic levels drop to normal spontaneously and without ill effect to the infant. In another newborn infant polycythemic levels with a maximum of 7.5 million red cells per cubic millimeter persisted for six weeks before a spontaneous drop to 5 million red cells occurred.[*] Erythremia or polycythemia vera is not known to occur in infancy.

Hematocrit Percentage (Volume of Packed Red Cells). The hematocrit obtained after centrifugation of a given amount of blood averages 55 per cent at birth, declines to 30 per cent by the second month, increases to 36 per cent at 1 year of age and 40 per cent at 3 years of age, and achieves normal adult values of about 45 per cent for males and 42 per cent for females in adolescence. Normal

children occasionally reveal a marked elevation of hematocrit red cells and hemoglobin during adolescence These changes simulating polycythemia are transient and benign revert to normal in the post adolescent period and require no treatment

Size of Red Cells (MCV) and Hemoglobin Concentration (MCHC) The red cells are unusually large at birth with an average in the cord blood of 113 cubic microns and a range of 90 to 124 cubic microns [6] They diminish in size to a value of 69 cubic microns at 1 year of age [9] The lower limit of normal of 80 cubic microns for children and adults is reached at 4 to 5 years [6] The adult MCHC of 34 per cent is reached at 4 years of age

Reticulocytes According to Windle [7] there are about 90 per cent reticulo cytes in the blood of the human fetus at 3 months gestation and 15 to 30 per cent at 6 months gestation The increased number of reticulocytes at birth reflects active red cell formation existing in fetal life

Reticulocytes number 4 to 6 per cent at birth and remain approximately con stant for about three days *after* birth *From the first half of the fourth day* to the first half of the sixth day of life there is a pronounced drop From the sixth to the seventh day there is a further and slower decrease to approximately adult levels (0 5 to 1 5 per cent) [18]

Normoblasts Normoblasts are frequently observed in the normal infant on the first day of life but usually disappear during the first week in most instances from the third to the fifth day The average number at birth ranges from 3 to 10 per 100 white blood cells In the premature infant the number of nucleated cells in the cord blood range from 10 to 20 per 100 white blood cells with higher figures prevailing in the smaller infant Occasional nucleated red cells will be observed in the peripheral blood of an older infant with acute infection associ ated with anemia The presence of normoblasts in the blood of the older infant and child with leukopenia granulocytopenia and moderate anemia is indicative of bone marrow embarrassment and frequently invasion of the marrow by leukemic cells

Platelets Variable numbers of platelets have been given for the neonatal period Some workers have found fewer platelets during the first forty eight hours than in older infants Figures reported at time of birth range from 150 000 to 350 000 per cubic millimeter with an average of 300 000 at 2 weeks of age The smaller number of platelets at birth has been attributed to the trauma incident to delivery The rise in platelets is supposedly slower in the premature infant The platelets of the newborn baby also show greater variation in size and shape than those in adults in whom larger platelets are found in appreciable numbers The adult values of 250 000 to 350 000 per cubic millimeter of blood are reached at about 6 months of age

White Blood Cells The total leukocyte count is high at birth ranging from 9 000 to 38 000 per cubic millimeter during the first two days of life with an average of 22 000 cells at the end of twelve hours [1] In the first week the white blood count drops to an average of 12 000 per cubic millimeter with a range of 5 000 to 21 000 At the end of the first year the white blood cell count averages 12 000 with a gradual decline to values of 8 000 to 10 000 by the fourth year The

total white blood cell count at birth tends to be lower in the premature infant than in the full term infant

Neutrophilic Leukocytes Neutrophilic leukocytes average 60 per cent at birth with a range from 40 to 80 per cent and include a small percentage of myelocytes By the tenth day the average drops to 40 per cent and then to 30 per cent in the fourth to the sixth month It remains at this level until the fourth year when a rise to 40 per cent takes place In the sixth year there is a rise to 55 to 60 per cent

Lymphocytes Lymphocytes average 30 per cent at birth and rise to 60 per cent in the fourth to sixth month These values are maintained until the fourth year when they drop to 50 per cent They drop to 40 per cent at the end of the sixth year and to 30 per cent by the eighth year

Monocytes Monocytes number 6 per cent at birth and except for a rise to 9 per cent in the second and third weeks remain at levels of about 5 per cent during infancy and childhood

Eosinophils and Basophils Eosinophils and basophils maintain levels of 2 to 3 per cent and 0 5 per cent respectively throughout infancy and childhood

Plasma Cells These are large spherical cells with deep blue cytoplasm containing vacuoles of varying size The nucleus eccentrically placed is round or oval with dense chromatin masses arranged like spokes in a wheel Plasma cells are not found in the embryo but appear after birth in interstitial lymphoid and glandular tissue Clinical evidence suggests their relationship to immunity because of their presence in patients with subacute and chronic infections Increased numbers of plasma cells have been noted in patients with infections in whom hyperglobulinemia is found On the other hand patients with agammaglobulinemia regularly exhibit a deficiency of plasma cells in hematopoietic centers and in inflammatory exudates [1]

Summary of White Blood Cell Changes Certain approximate values may be designated for comparative purposes At the end of the second month of life leukocytes total 12 000 per cubic millimeter with 35 per cent granulocytes and 60 per cent lymphocytes These values are maintained until the fourth and fifth years when the total count drops to 8 000 white cells per cubic millimeter and the differential percentage is reversed to 60 per cent granulocytes and 35 per cent lymphocytes From the sixth to the fourteenth year there is a gradual numerical shift to adult values of 7 000 white blood cells per cubic millimeter with 65 per cent neutrophilic granulocytes and 30 per cent lymphocytes Newborn infants with total leukocyte counts at either extreme should be investigated for existing abnormalities There is evidence that premature infants as compared with full term infants show higher granulocyte lower lymphocyte and lower white blood cell counts

Blood of Premature Infants At the end of the decline in the second and third months the premature infant shows hemoglobin levels of 1 gm or more (depending upon the birth weight) below the values of 11 gm for full term infants and red blood cell counts below the average of 3 800 000 for full term infants Thereafter a gradual recovery sets in with values approximating normal full term levels at about 1 year of age The number of platelets may be somewhat lower at birth

in the premature infant than in full term infants averaging about 190 000 per cubic millimeter at birth and rising to 300 000 at the sixth month The total number of white cells may be lower and nucleated red cells may persist for longer periods than in the full term infant *Polycythemia at birth may be exaggerated* in the premature infant as compared with the full term infant because of differences in the proportion of the volume of blood in the body to that in the placenta Whereas the amount of blood in the placenta remains fairly constant during the third trimester of pregnancy that in the body increases steadily The effect of the transfer of placental blood in the infant after birth therefore would produce a greater relative effect in the premature infant than in the full term infant especially when tying of the cord is delayed and the full complement of blood is obtained from this source

REFERENCES

1 Barr D P The Function of the Plasma Cell Am J Med 9 277 1950
2 DeMarsh Q B Windle W F and Alt A L Blood Volume of Newborn Infant in Relation to Early and Late Clamping of Umbilical Cord Am J Di Child 63 1123 1942
3 Gairdner D Marks J and Roscoe J D Blood Formation in Infancy Normal Erythropoiesis Arch Dis Childhood 27 214 1952
4 Gairdner D Marks J Roscoe J D and Brettell R O The Fluid Shift From the Vascular Compartment Immediately After Birth Arch Dis Childhood 33 489 1958
5 Good R A Agammaglobulinemia Bull Univ Minnesota Hosp 26 1 1954
6 Guest G M Brown E W and Wing M Erythrocytes and Hemoglobin of the Blood *in Infancy and Childhood Variability in Number Size and Hemoglobin Content* of the Erythrocytes During the First Five Years of Life Am J Dis Child 56 529 1938
7 Guest G M and Brown E W Erythrocytes and Hemoglobin of the Blood in Infancy and Childhood Factors in Variability Statistical Studies A M A J Dis Child 93 486 1957
8 Haselhorst G and Allmeling A Die Gewichtszunahme von Neugeborenen infolge postnataler Transfusion Ztschr Geburtsh u Gynak *98 103 1930*
9 Horan M Studies in Anemia of Infancy and Childhood the Hemoglobin Red Cell Count and Packed Cell Volume of Normal English Infants During the First Year of Life Arch Dis Childhood 25 110 1950
10 Kato K Leucocytes in Infancy and Childhood J Pediat 7 7 1935
11 Klingberg W G Jones B Allen W M and Dempsey E Placental Parabiotic Circulation of Single Ovum Human Twins A M A Am J Dis Child 90 519 1955
12 Leichsenring J M Norris L M and Halbert M L Hemoglobin Red Cell Count and Mean Corpuscular Hemoglobin of Healthy Infants A M A Am J Dis Child 84 27 1952
13 Lippman H S A Morphologic and Quantitative Study of the Blood Corpuscles in the New Born Period Am J Dis Child 27 473 1924
14 Merritt K K and Davidson L T The Blood During the First Year of Life Am J Dis Child 46 990 1933
15 Mugrage E R and Andresen M I Values for Red Blood Cells of Average Infants and Children Am J Dis Child 51 775 1936
16 Mollison P L and Cutbush M Haemolytic Disease of the Newborn In Gairdner Douglas (editor) Recent Advances in Pediatrics New York 1954 Blakiston Co
17 Schulman I *Smith C H and Stern G S Studies on the Anemia of Prematurity* A M A Am J Dis Child 88 567 1954
18 Seip M The Reticulocyte Level and the Erythrocyte Production Judged From Reticulocyte Studies in Newborn Infants During the First Week of Life Acta paediat 44 355 1955
19 Shapiro L M and Bassen F A Sternal Marrow Changes During the First Week of Life Correlation With *Peripheral Blood Findings Am J M Sc* 202 341 1941

20 Smith C H Hypoplastic and Aplastic Anemias of Infancy and Childhood With a Consideration of the Syndrome of Nonhemolytic Anemia of the Newborn J Pediat 43 457 1953
21 Albritton E C (editor) Standard Values in Blood Biological Data AF Technical Report No 6039 1951
22 Tocantins L M Mammalian Blood Platelet in Health and Disease Medicine 17:155 1938
23 Washburn A H Blood Cells in Healthy Young Infants The Leukocyte Picture During the First Three Months With Special Reference to Hourly and Daily Variations Am J Dis Child 47:993 1934
24 Washburn A H Blood Cells in Healthy Young Infants a Comparison of Routine and Special Technics in the Differentiation of Leukocytes Am J Dis Child 50 395 1935
25 Washburn A H Blood Cells in Healthy Young Infants a Study of 608 Differential Leukocyte Counts With a Final Report on 908 Total Leukocyte Counts Am J Dis Child 50 412 1935
26 Whipple C A Sisson T R C and Lund C J Delayed Ligation of the Umbilical Cord Obst & Gynec 10 603 1957
27 Windle W F Development of the Blood and Changes in the Blood Picture at Birth J Pediat 18 538 1941
28 Lichtman I Laboratory Applications in Clinical Pediatrics New York 1957 Blakiston Co
29 Wood J L Plethora in the Newborn Infant Associated With Cyanosis and Convulsions a Review of Postnatal Erythropoiesis J Pediat 54 143 1959

CHAPTER 3

Blood Dyscrasias in Relation to Maternal-Fetal Interaction

The pathogenesis and interpretation of many of the blood dyscrasias characteristic of infancy and childhood depend upon a consideration of maternal fetal relationships, environmental and genetic influences, and normal developmental changes. The problems of diagnosis and therapy often hinge on an understanding of many of the interrelated mechanisms that play a role in the control of the formation and development of blood cells.

Hereditary Basis of Blood Diseases—Genetic and Environmental Influences Many of the blood disorders in the pediatric age group are conditioned by the overlapping influence of heredity and environment. Congenital defects may be genetically determined at the moment of conception or acquired during fetal development due to stress in the mother during critical moments of gestation. Warkany[4, 43] postulated that the repeated appearance of defects in a family indicates the influence of a dominant or recessive inheritance of abnormal genes or of the repeated exposure of the developing embryo to the same adverse environmental factors. The majority of developmental defects are caused by a combination of circumstances involving both genetic and environmental factors.[11, 1]

Blood Dyscrasias in Relation to Congenital Anomalies—General Principles A growing body of evidence has been accumulating[1] in support of the association of a variety of disease states in the mother and the occurrence of abnormalities in the offspring. In the elaboration of these concepts more intensive investigation has also been directed toward genetic and familial aspects of a variety of diseases in which many of the blood disorders have been included.

The experimental production of developmental defects has prompted consideration of the origin of anomalies in every system. According to current interpretation, single or co-existing anomalies represent deviations from orderly development induced by a known infection such as rubella or other as yet unidentified agents or states acting upon the pregnant woman and the placenta. If one accepts the principle that for all organs injury is most likely to occur during the stage of active differentiation, the vulnerable period of erythropoiesis would extend from the sixth to the twelfth week when the liver, spleen, and

bone marrow are simultaneously involved in the proliferation of erythroblasts Chronic thrombocytopenia present from birth may be related to suppression of platelet formation from the second month of fetal life when platelet production becomes evident [11]

It is thus apparent that the type of deformity depends upon nonspecific noxious influences operating at "developmental moments" corresponding to periods when organs are in their most rapidly proliferating condition The infrequent association of severe types of hematologic disorders [79] with other congenital malformations suggests the relative resistance of hematopoietic tissues in comparison with the vulnerability of other systems during critical periods of development However the demonstration of injury to single or multiple cell types in the marrow in combination with other well-defined somatic defects *infrequent as it appears* supports the hypothesis of embryonic hematopoietic disturbances as a causative factor in blood dyscrasias

Examples of blood dyscrasias associated with congenital anomalies include congenital leukemia and mongolism hypoplastic aplastic anemias with skeletal defects (that is Fanconi syndrome) sporadic congenital spherocytosis associated with congenital hypoplastic thrombocytopenia and malformations and congenital labile factor deficiency with syndactylism

Hematologic Aspects of Maternal and Fetal Interaction—Placental Physiology and Defects The bulk of the placenta consists of chorionic villi which are exposed to a collection of maternal blood Fetal and maternal blood are separated from each other by only two layers of cells at the trophoblastic endothelial junction of each villus The maternal blood constantly circulates between the individual villi from which it is drained by the decidual sinuses Microscopic defects or breaks in the villi conceivably could permit leaks of small amounts of fetal blood into the maternal circulation causing mild to moderate anemia in the newborn infant [1] as may be observed in Fig 1

Maternal arterial blood enters the intervillous space at a pressure of 60 to 70 mm Hg higher than the already existing pressure in this area and is dissipated by lateral dispersion On the other hand the difference between the umbilical arterial blood pressure and that in the intervillous space creates a pressure gradient conducive to the passage of water and metabolites from fetus to mother It is conceivable that fetal blood may follow a similar course especially through breaks or infarcts in the villi resulting in neonatal anemia exclusive of incompatibilities [16] These observations suggest a mechanism by which an Rh negative woman is sensitized by an Rh positive fetus (namely by the transplacental passage of fetal red blood cells or their products)

Fetal Hemorrhage Into the Maternal Circulation (Nonhemolytic Anemia of the Newborn Infant) Wiener[4] was the first to suggest that the fetus can become severely anemic from bleeding into the maternal circulation This concept was confirmed by Chown [6] who reported a case in which a high concentration of fetal hemoglobin as well as fetal red cells could be found in the maternal blood soon after delivery This suggested to him that anemia in the infant resulted from bleeding into the maternal circulation The subsequent disappearance of fetal hemoglobin from the maternal circulation coincided with the disappearance

of the infant's D (Rh_0) positive cells It was calculated that approximately 90 to 180 ml of fetal blood had entered the maternal circulation sometime before birth

When transplacental bleeding from the fetus occurs appropriate agglutination and serologic tests reveal that the mother has been the recipient of erythrocytes which differ in the ABO and Rh blood groups from her own erythrocytes and which are identical with those of the newborn infant Also an elevated concentration of bilirubin and fetal hemoglobin in the mother's circulation immediately postpartum and their subsequent disappearance constitute further proof of the

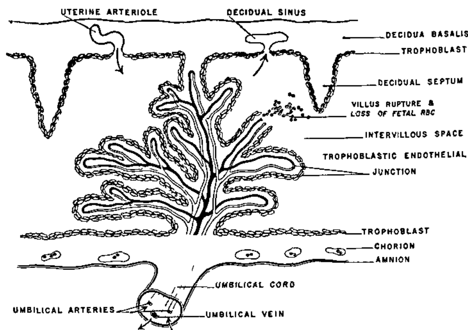


Fig 1 Schematic diagram of the placental circulation showing rupture of a villus at the trophoblastic endothelial junction permitting the mixing of fetal and maternal blood and the entrance of fetal red blood cells into the maternal circulation (Modified from Javert C T and Reiss C Origin and Significance of Macroscopic Intervillous Coagulation Hematomas [Red Infarcts] of Human Placenta Surg Gynec & Obst 94 257 1952)

passage of fetal blood into the maternal circulation through breaks of varying size in the placental barrier The finding of increased fetal hemoglobin levels in the mother's blood which gradually diminish provides evidence for the leakage of fetal blood when the ABO and Rh blood groups in the fetus and the mother are identical [3]

Additional evidence for the entrance of fetal antigens into the maternal circulation just before delivery is the reported appearance in group O mothers of immune anti A antibodies (from A infants) ten to twenty days after delivery when none had been present at birth [10]

The loss of fetal blood into the maternal circulation has been described in

many reports on the basis of an excess of alkali resistant hemoglobin and differential agglutination of red cells in maternal blood[21, 18, 23, 24] Iron deficiency anemia has been reported as one of the complications of occult blood loss due to fetal maternal transfusion [4]

Symptomatology and Blood Findings An evaluation of these reports indicates that the leakage of fetal blood into the maternal circulation can occur in small amounts over a prolonged period or may take place in larger amounts shortly before delivery or during labor Severe anemia and shock in the immediate postnatal period indicate transplacental hemorrhage from fetus to mother just prior to delivery[28] Pallor is a significant feature jaundice is absent The spleen and liver are not enlarged Anemia of moderate or severe degree with a hypochromic microcytic blood smear characteristic of iron deficiency may be present If severe hemorrhage occurs at the time of delivery the cord blood hemoglobin may be normal due to hemoconcentration The evidences of anemia become perceptible with later hemodilution Leukocytosis reticulocytosis and nucleated red cells are noted occasionally but hyperbilirubinemia is unusual in contrast to erythroblastosis[1]

The mother may experience no symptoms during pregnancy from fetal bleeding into her circulation When the mother is the recipient of incompatible blood from the fetus the symptomatology is analogous to a transfusion reaction with chills fever and hyperbilirubinemia [1]

Treatment Transfusions with packed cells sufficient to bring the hemoglobin to approximately 12 gm per 100 ml are advisable in severe anemia Since the loss of blood implies a decrease of potential iron stores supplemental oral iron medication is advisable during recovery

Bleeding From the Placental Surface A significant number of newborn infants are born anemic not only because of occult hemorrhage from the passage of fetal blood into the maternal circulation but also because of serious fetal blood loss during labor and delivery In abruptio placentae and placenta previa bleeding takes place from the placental surface in the area of its attachment to the uterine wall Blood loss suffered by the mother is shared by the infant with resulting posthemorrhagic anemia and shock (asphyxia pallida) and even stillbirth Similar consequences follow intrauterine rupture or tear of fetal vessels during labor[27] Exsanguination from rapid blood loss occurs in precipitous deliveries from velamentous insertion of the cord and less commonly from rupture of a normal or abnormally short cord[19, 20] Treatment is urgent and consists of clearing the airway and administering oxygen artificial respiration and transfusions with packed red cells

Placental Transmission of Antibodies and Isoagglutinins Since from an anatomic point of view the maternal and fetal circulations in man are separated by only two layers of cells placental transmission of a variety of substances including maternal antibodies isoagglutinins plasma proteins and steroids is effected in the human infant Antibodies and gamma globulin are not produced in significant amounts in the fetus of any species under normal conditions Their presence in the fetus and newborn infant prenatally results from transfer across the placenta from the mother Postnatally these substances are derived from the

of the infants D (Rh_0) positive cells It was calculated that approximately 90 to 180 ml of fetal blood had entered the maternal circulation sometime before birth

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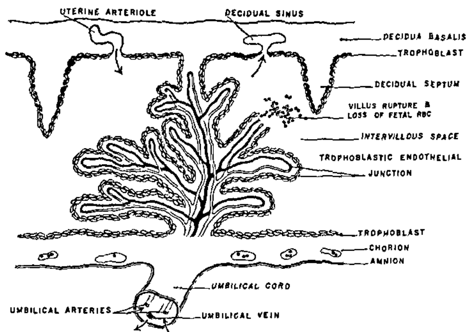


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The loss of fetal blood into the maternal circulation has been described in

with the number of cases in which the factor presumably appears in the newborn infant by passive transfer

Plasma Proteins in the Fetus and Newborn Infant The gamma globulin in the cord blood and in the blood of the newborn baby (600 to 1,200 mg per 100 ml) is derived entirely from the maternal circulation by placental passage Low gamma globulin levels are found in fetal blood until the fourth or fifth month of pregnancy after which they rise gradually to reach maternal concentrations by the eighth to ninth month At birth the gamma globulin level in fetal blood is somewhat higher than in maternal blood [41] The gamma globulin is observed to fall steadily in the first month of life following a simple exponential curve reaching one third of the birth value at 1 month of age when it is stabilized [3-4] No change occurs between 1 and 3 months of age (300 to 600 mg per 100 ml) when the rate of synthesis balances the rate of catabolism From 3 months of age levels of gamma globulin rise slowly and progressively and reach adult levels (900 mg per 100 ml) at 2 years of age [1] According to these calculations the half life of gamma globulin is approximately twenty five days [33 34] which is comparable to the thirty-day half life of the Rh antibody molecule which passes through the placenta [44]

Dancis and associates have shown that under normal circumstances the placenta does not contribute significantly to the plasma proteins of the fetus Beginning early in gestation the fetal liver is capable of synthesizing all plasma proteins with the exception of gamma globulin Little gamma globulin can be detected in fetal plasma up to six months of gestation [30] It is problematic whether the infant normally synthesizes gamma globulin before the fourth week of extrauterine life Probably no significant amounts are synthesized before the age of 3 months

Mean values for total serum protein are lower in the cord blood of newborn infants than in their mothers at term The lowering is a reflection of the lower globulin fraction since the mean value for serum albumin is much higher in the newborn infant than in the mother at term After birth the level of serum globulin falls steadily reaching its lowest level at 3 to 4 months of age and then rising to adult levels by 7 to 11 months of age [31]

Immunologic Relationships The following immunologic relationships exist

Erythroblastosis Fetalis The maternal fetal relationships in relation to sensitization by antigens of the Rh positive fetus and in cases of a heterospecific pregnancy of the A B and O groups will be dealt with in another chapter (Chapter 9) They represent important examples of antibody relationships and immunosensitization between maternal and fetal blood

Thrombocytopenic Purpura in the Newborn Infant Hemorrhagic disease of the newborn infant and congenital thrombocytopenic purpura make their appearance soon after birth and reflect disturbances resulting from maternal fetal relationships Thrombocytopenic purpura in the newborn infant may be cited as another example of the influence of maternal suppressing factors on a fetal blood element This disease affects children whose mothers either have thrombocytopenic purpura or are entirely normal In the former instance the reduction of platelets in the infant results from the passage across the placental membrane

ingestion of colostrum and maternal milk.[41] This contrasts with the more difficult transmission in the ruminant in which there is a complex placental structure (five tissue layers between maternal and fetal circulation at full term of gestation). Therefore, as knowledge of placental function has been extended, it has become increasingly clear that a wide variety of substances, including *proteins* and red cells, cross the placental barrier into the fetal circulation and also that the rate of transfer is selectively controlled.[9]

While A and B isoagglutinogens can be demonstrated in the fetus in the second and third months,[7, 22] isoagglutinins appear later. In a majority of newborn infants isoagglutinins detected in the cord blood diminish in titer or completely disappear. Consideration of the similarity existing between the blood of the baby and that of the mother with respect to isoagglutinins suggests the passive transfer of isoagglutinins from the maternal into the fetal circulation through the placenta.[30] This process is analogous, for example, to the transplacental passage of anti-Rh antibodies, gamma globulin, diphtheria and tetanus antitoxin and immune bodies for measles and mumps virus from mother to child.

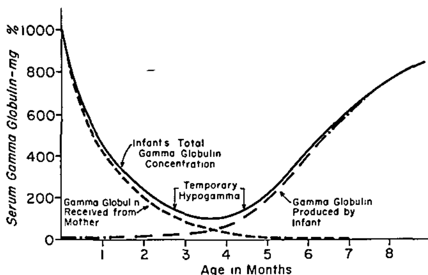


Fig. 2. Graph showing levels of gamma globulin in physiologic hypogammaglobulinemia of newborn infants. As level of gamma globulin from mother drops and production by infant begins, there is a temporary drop in gamma globulin concentration. (From Barrett, B., and Volwiler, W.: Agammaglobulinemia and Hypoglobulinemia. J.A.M.A. 164:866, 1957.)

Placental Transmission of the L.E. Factor. Cases of systemic lupus erythematosus in pregnancy have been reported in which the L.E. phenomenon has been demonstrated in the newborn infant.[4, 8] The L.E. factor was present in the cord blood and persisted for approximately seven weeks after delivery, a period corresponding with the half life of gamma globulin with which it is identified. (See discussion on L.E. phenomenon, Chapter 18.) The babies remained healthy; this was considered an indication that the L.E. factor is derived by transplacental passage from the mother rather than by an immunologic process developing in utero. The inheritance of systemic lupus erythematosus is rare[14] in comparison

since it cannot be replenished once the maternal source is eliminated except in circumstances in which it can be conveyed by colostrum or maternal milk

REFERENCES

1 Bass M In Dock W and Snapper I (editors) Advances in Internal Medicine vol 5 Chicago 1952 Year Book Publishers Inc
2 Berlyne G M Short I A and Vickers C F H Placental Transmission of the LE Factor Lancet 2 15 1957
3 Borum A Lovd H O and Talbot T R Jr Possible Fetal Hemorrhage Into Maternal Circulation J A M A 164 1087 1957
4 Bridge R C and Foley F E Placental Transmission of the Lupus Erythematosus Factor Am J M Sc 2.7:1 1951
5 Chown B Anaemia in a Newborn Due to the Fetus Bleeding Into the Mother s Circulation Proof of the Bleeding Lancet 1 1213 1951
6 Chown B Anaemias in the Newborn Other Than Haemolytic Disease Pediat Clin North America 4 371 1957
7 Congenital Malformations Pediatrics (suppl) 23 195 1959
8 Dancis J Braverman N and Lind J *Plasma Protein Synthesis in the Human Fetus* and Placenta J Clin Invest 36 398 1957
9 Dancis J The Placenta J Pediat 55 85 1959
10 Dunsford I Proof of Fetal Antigens Entering the Maternal Circulation Official Program of the Sixth International Congress of the International Society of Hematology Boston 1956 p 539
11 Fraser F C and Fainstat T D Causes of Congenital Defects a Review A M A Am J Dis Child 82 593 1951
12 Fraser F C Walker B E and Trasler D G Experimental Production of Congenital Cleft Palate Genetic and Environmental Factors Pediatrics 19 782 1957
13 Fruhling L Roger S and Jobard P L hematologie normale (tissus et organes hematopoietiques sang circulant) de l embryon du foetus et du nouveau ne humains Sang 20 267 1949
14 Gligov S and Cechman F Familial Occurrence of Disseminated Lupus Erythematosus New England J Med 255 936 1956
15 Goodall H B Graham F S Miller M D and Cameron C Transplacental Bleeding *From the Foet s* J Clin Path 11 251 1958
16 Gunson H H Neonatal Anemia Due to Fetal Hemorrhage Into the Maternal Circulation Pediatrics 20 3 1957
17 Janeway C A and Gitlin D The Gamma Globulins In Levine S Z and others (editors) Advances in Pediatrics vol 4 Chicago 1951 Year Book Publishers Inc p 65
18 Javert C T and Reiss C The Origin and Significance of Macroscopic Intervillous Coagulation Hematomas (Red Infarcts) of the Human Placenta Surg Gynec & Obst 94 257 1952
19 Kirkman H N and Riley H D Posthemorrhagic Anemia and Shock in the Newborn Due to Hemorrhage During Delivery Report of 8 Cases Pediatrics 24 92 1959
20 Kirkman H N and Riley H D Posthemorrhagic Anemia and Shock in the Newborn a Review *Pediatrics 24 97 1959*
21 Levine P Serological Factor as Possible Causes in Spontaneous Abortions J Hered 34 71 1943
22 Levine P Burnham L Katzin F M and Vogel P The Role of Isoimmunization in the Pathogenesis of Erythroblastosis Fetalis Am J Obst & Gynec 42 295 1941
23 Lucey J F and Dolan R G Injections of Vitamin K *Compound in Mothers and* Hyperbilirubinemia in the Newborn Pediatrics 22 605 1958
24 Luhby A L and Slobody L B Transient Neonatal Agranulocytosis in Two Siblings Transplacental Isoimmunization to a Leucocyte Factor? A M A J Dis Child 92 496 1956

of a circulatory factor which depresses platelet formation in the infant and causes purpura. Platelet autoagglutinins have been found in the blood of the mother and infant. Isoagglutinins for each other's platelets have been found also.[3] These agglutinins disappear from the infant's circulation by the age of 3 months.

Fetal Maternal Leukocyte Incompatibility. That leukoagglutinins may develop in multitransfused patients has been recognized. Leukoagglutinins are believed to be comparable to antibodies for erythrocytes and platelets. Antigenic differences in human leukocytes are known and leukoagglutinins have been held responsible for febrile transfusion reactions.[33] Fetal maternal incompatibility may induce leukoagglutinin formation in the mother who has had no transfusions when the antigenic factor has been inherited from the father and is absent in the mother.[34]

Transient agranulocytosis has been observed in successive siblings in the neonatal period.[1] Such a circumstance was explained by transplacental isoimmunization of the mother to a leukocyte factor of her infant in a manner analogous to Rh isoimmunization causing hemolytic disease of the newborn infant. In these cases agranulocytosis persisted for three to four weeks and in one of the infants it was accompanied by pulmonary infection. The predominance of neutrophilic myelocytes in the bone marrow in these infants may represent either a maturation arrest or a depletion of mature cells because of their increased agglutination and destruction in the peripheral blood.

Two infants born of mothers with chronic neutropenia showed a transitory neutropenia which persisted for three weeks.[40] The blood picture in the babies was presumably caused by the transplacental passage of a neutropenic factor. In one case the mother's serum contained a demonstrable leukoagglutinin which could be transferred by transfusion to a normal person. In these patients the bone marrow showed hyperplasia of the granulocytic series with a maturation arrest at the myelocytic stage.

Transplacental Passage of Drugs Affecting Blood Elements in the Newborn Infant. Several examples may be cited of the passage of drugs through the placenta affecting the blood elements in the newborn infant. In one case report the administration of quinine to induce labor resulted in thrombocytopenia in the mother and infant. The mechanism was based on the transmission of quinine platelet antibodies derived from the mother who had originally received the drug in childhood. The parenteral administration of a large dose of a vitamin K analogue (Hykinone) to mothers during labor has also been shown to result in marked hyperbilirubinemia in newborn premature infants with nervous system involvement in several cases.[3] The increased pigment presumably resulted from the hemolytic effects of the vitamin. In another case[46] the ingestion of moth balls by a mother produced jaundice and hemolytic anemia in her newborn infant. This was attributed to the passage of naphthalene and its derivatives to the infant.

Generally substances capable of injuring the fetus and newborn infant which have been transmitted by transplacental passage from the mother play a passing role in producing pathologic states. The noxious agent has a limited activity

CHAPTER 4

Erythrocytes—General Considerations

Properties of the Erythrocyte The essential morphologic and physiologic aspects of the erythrocyte are as follows

Erythron The term erythron refers to the tissue made up of the circulating red blood cells and their precursors in the bone marrow It conveys a sense of functional unity to a series of cells ranging from the early erythroblast to the non nucleated red corpuscles

Erythrocytes–Structure and Function Normal human red blood cells are non nucleated biconcave discs whose primary function is the transport of hemoglobin which represents 34 per cent of their fresh weight They are structurally adapted for rapid movement throughout the circulation to bring oxygen from the lungs and distribute it rapidly to the tissues through the smallest capillaries The red cell is thicker at the periphery than in the central portion and possesses an elasticity which facilitates mechanical buffetting and movement through capillaries narrower than its own diameter Collectively the red cells offer an extensive surface of approximately 3 000 square meters in the adult to facilitate the absorption and discharge of oxygen and perhaps contribute to the movement of other substances by surface absorption Hemoglobin is not held in solution but adheres closely within the meshes of stroma a spongelike material filling the interior of the cell The outer surface or envelope which serves as a semipermeable selective membrane in the passage of substances in and out of the cell is composed principally of lipids consisting of a protein stromatin in combination with lecithin and cholesterol The red cell surface possesses blood group properties affinities and specificities involved in hemolysis and agglutination

In addition to hemoglobin which constitutes more than 90 per cent of solids the red cell contains water protein liquids carbohydrates vitamins iron and a group of enzyme systems which maintains the integrity of the cell membrane In the circulation hemoglobin remains relatively static whereas other red cell constituents undergo constant physical and chemical changes relating to their metabolism[18]

It has been pointed out that in addition to the structural proteins there are approximately forty enzymes thus far identified in the human red cell[60] Among these are the specific enzymes

25 Mauer A M DeVaux W and Lahey M E Neonatal and Maternal Thrombocytopenic Purpura Due to Quinine Pediatrics 19 84 1957
26 McGovern J J Driscoll R DuToit C H Grove-Rasmussen M and Bedell R F Iron Deficiency Anemia Resulting From Fetomaternal Transfusion New England J Med 258 1149 1958
27 Michaels J P Intrauterine Rupture of Fetal Vessels During Labor Am J Obst & Gynec 70 1251 1955
28 Myer F and Olsen R N Transplacental Passage of the L E Factor J Pediat 52 690 1958
29 Miller H C Clifford S H Smith C A Warkany J Wilson J L and Yannet H Special Report From the Committee for the Study of Congenital Malformations of the American Academy of Pediatrics Study of the Relation of Congenital Malformations to Maternal Rubella and Other Infections Preliminary Report Pediatrics 3 259 1949
30 Moore D H Martin du Pan R and Buxton C L An Electrophoretic Study of Maternal Fetal and Infant Sera Am J Obst & Gynec 57 312 1949
31 Oberman J W Gregory K O Burke F G Ross S and Rice E C Electrophoretic Analysis of Serum Proteins in Infants and Children Normal Values From Birth to Adolescence New England J Med 255 743 1956
32 O Connor W J Shields G and Schuyler K Anemia of the Newborn in Association With the Appearance of Fetal Hemoglobin in Maternal Circulation A M A J Dis Child 93 10 1957 (Abst)
32a Orlandini O Sass Kortsak A and Ebbs J H Serum Gamma Globulin Levels in Normal Infants Pediatrics 16 575 1955
33 Payne R *The Association of Febrile Transfusion Reactions With Leuko-agglutinins* Vox Sang 2 233 1957
34 Payne R and Rolfs M R Fetomaternal Leukocyte Incompatibility J Clin Invest 37 1756 1958
35 Pearson H A and Diamond L K Fetomaternal Transfusion A M A J Dis Child 97 267 1959
36 M & R Laboratorie Report of the Fifteenth M & R Pediatric Research Conference 1955 p 27
37 Schoen E J King A L and Duane R T Neonatal Thrombocytopenic Purpura Pediatrics 17 72 1956
38 Shiller J G Shock in the Newborn Caused by Transplacental Hemorrhage From Fetus to Mother Pediatrics 20 7 1957
39 Smith C H Iso agglutinins in the Newborn With Special Reference to Their Placental Transmission Am J Dis *Child* 36 54 1928
40 Stefanini M Mele R H and Skinner D Transitory Congenital Neutropenia a New Syndrome Report of Two Cases Am J Med 25 749 1958
41 Vahlquist B Transfer of Antibodies From Mother to Offspring In Levine S Z and others (editors) Advance in Pediatrics vol 10 Chicago 1958 Year Book Publishers Inc p 305
42 Warkany J Etiology of Congenital Malformations In Levine S Z and other (editors) Advances in Pediatrics vol 2 New York 1947 Interscience Publishers Inc p 1
43 Warkany J Congenital Anomalies Pediatric 7 607 1951
44 Wiener A S The Half Life of Passively Acquired Antibody Globulin Molecules in Infants J Exper Med 94 213 1951
45 Wiener A S Diagnosis and Treatment of Anemia of Newborn Caused by Occult Placental Hemorrhage Am J Obst & Gynec 56 717 1948
46 Zinkham W H and Childs B *A Defect of Glutathione Metabolism in Erythrocyte* From Patients With a Naphthalene Induced Hemolytic Anemia Pediatrics 22 461 1958

Porphyrins and Blood Disorders The porphyrins are red pigments with a pyrrole structure which serve as precursors of protoporphyrins I and III and of their corresponding coproporphyrins. Both coproporphyrins are excreted in excess in urine and stool in certain blood disorders: type I in pernicious anemia, congenital spherocytic anemia and leukemia, and type III in aplastic anemia and Hodgkin's disease, and after administration of heavy metals such as mercurials and arsenicals. The examination of the urine for porphyrins is especially important in lead poisoning. The greatest increases in coproporphyrin excretion occur in this condition, although this may not be a consistent finding. Splenectomy may be followed by diminished porphyrin formation in the bone marrow. Free protoporphyrin, normally found in small amounts in erythrocytes, is greatly increased in the blood of patients with iron deficiency anemia and disorders in which utilization of iron in hemoglobin synthesis is interfered with, such as in anemia of infection.

Rouleaux Formation and Sedimentation In a fresh preparation on a slide red cells tend to cluster together with their broad surfaces in contact, forming a grouping called rouleaux. The disproportionate thickness and difficulty of approximation of the red cells in congenital spherocytic anemia results in loss of rouleaux formation and represents a diagnostic feature of this disease.

In conditions associated with rapid sedimentation the initial phase consists of increased rouleaux formation: the larger the aggregates, the more rapid is the sedimentation rate. Accelerated rates are primarily influenced by increases in fibrinogen and, to a large extent, by globulin in the plasma.

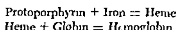
Electrolyte Considerations The electrolyte composition of red blood cells is qualitatively similar to the plasma, except for the predominance of potassium in the former and sodium in the latter. In the passage of inorganic ions in and out of the cell, the red cell membrane acts as a dynamic rather than as a passive semipermeable structure. The energy for this and other functions of the mammalian cell is derived from the breakdown of glucose into lactic acid and from the presence of nucleosides such as adenosine triphosphate (ATP). In normal metabolic activity of the red cell membrane, a high concentration of potassium is maintained within the cell and sodium is excluded. In blood stored under refrigeration, glycolytic activity is inhibited and potassium diffuses out of the cells into the plasma and sodium enters.[40] The accumulation of potassium in the donor plasma is an important consideration in exchange transfusions in erythroblastosis and can conceivably result in increased serum potassium levels in the infant. With normal kidney function, however, this state rarely develops. On the other hand, the too rapid infusion of hyperkalemic donor blood that is not fully warmed carries the potentiality of untoward toxic effects. Since the metabolic activity is restored by exposure to 37° C and results in return of the intracellular potassium, the use of fresh warm blood is recommended.[6,34] It has also been found that the ability of stored red cells to regain potassium when they are warmed depends upon the presence of adenosine and that the addition of this substance and glucose is needed for optimal red cell preservation.[11,1]

Adsorption by the Surface of Red Blood Cells In the relation between the red cells and their environment, the occurrence of adsorptive phenomena is to be

linked with the multiple activities of the red cell in supplying energy from glycolysis. Another example of the mediation of enzymes is the increased susceptibility of the cell to hemolysis caused by primaquine sensitivity due to a low glutathione level of the red cell and a deficiency of glucose 6 phosphate dehydrogenase activity.

The normal red blood cell has a volume of 65 cubic microns and contains 28 to 30 micromicrograms of hemoglobin. The red cells, macrocytic at birth, diminish in size during the neonatal period and reach normal sizes of 7.2 to 7.5 microns in diameter by 8 months of age.

Hemoglobin—Structure and Function. Hemoglobin, the coloring matter of erythrocytes, *is a conjugate of a pigment (heme) and a protein (globin).* The heme component consists of a porphyrin (the union of four pyrrole groups) in combination with iron in the ferrous state. The hemes are pigments which account for the characteristic red color of hemoglobin. The particular porphyrin of hemoglobin is structurally a protoporphyrin. In the synthesis of heme, protoporphyrin is elaborated from the interaction of glycine and "active" succinate.[9] In vitro evidence suggests that the biosynthesis of heme from iron and protoporphyrin is enzyme dependent.[4] Hemoglobin may then be regarded as a ferrous protoporphyrin with the following composition:


Protoporphyrin + Iron = Heme
Heme + Globin = Hemoglobin

Heme is also found in myoglobin (muscle hemoglobin) and in the respiratory *enzymes such as the cytochromes. The basic function of the heme compounds* is to transport oxygen and make it available to the cell. Hemoglobin combines loosely with oxygen to form oxyhemoglobin; this unstable association permits the diffusion of oxygen to tissues for oxidative purposes. The iron of hemoglobin remains in the ferrous state throughout respiration. Methemoglobin, on the other hand, is the oxidized heme formed from reduced hemoglobin in which the iron is in firm combination in the ferric state and is incapable of functioning as an oxygen carrier. Globin, the protein component of the hemoglobin, is synthesized *from the amino acids of destroyed protein and from the globin of effete red blood* cells. For the complete synthesis of globin an adequate intake of protein is required.

Iron Content and Oxygen Capacity. Hemoglobin contains 0.335 per cent of iron so that 1 gm may be said to contain 3.4 mg of iron. Also, 1 gm of hemoglobin unites with 1.34 ml of oxygen. Oxygen capacity depends upon hemoglobin concentration. Since the normal hemoglobin in the older child and adult is approximately 15 gm, the oxygen capacity is therefore 20 ml per 100 ml of blood or 20 *volumes per cent.* The degree of oxygen saturation of blood is derived from the ratio of oxygen content (19 volumes per cent in arterial blood) to oxygen capacity and is normally 95 per cent.

Cyanosis depends upon *the absolute amount of reduced hemoglobin in capillary blood* and not upon the content of carbon dioxide. It has been determined that capillary blood must contain at least 5 gm of hemoglobin per 100 ml of blood to reveal cyanosis. In severe anemia with values below this level the amount of reduced hemoglobin will therefore be insufficient to show cyanosis.

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Adsorption by the Surface of Red Blood Cells. In the relation between the red cells and their environment, the occurrence of adsorptive phenomena is to be

expected The extensive surface presented by the red blood cells and its intimate contact with a diversity of substances in the plasma provide opportunities for adsorptive processes[43] comparable to those which occur at other interfaces This function of red blood cells as yet but little investigated may account in part for the transport of metabolic immune and endocrine products through the blood stream The quantity attached *to the red blood cell however is probably minimal* as compared with that free in the plasma and the substances fixed by the stroma are probably more slowly released than those in the plasma

Osmotic Fragility Changes in osmotic equilibrium surrounding the red cells influence their size At a concentration of 0 85 per cent a solution of sodium chloride is isotonic for the red cell so neither swelling nor shrinkage results In a medium of hypotonic sodium chloride solution on the contrary fluid passes into the cells increasing their size and the cells lose their biconcavity and become spherical As a critical level is reached more and more of the cells swell burst and release hemoglobin The first trace of hemolysis normally appears when so dium chloride concentration *reaches* 0 42 *per cent and hemolysis is complete at 0 32 to 0 35 per cent*

The resistance of the red corpuscles to varying dilutions of hypotonic solution of sodium chloride constitutes an important laboratory procedure in the diagnosis of the anemias and is especially useful in the differentiation of the hemolytic group The response of blood cells in the fragility test does not necessarily reflect their reaction within the circulating blood where conditions of isotonicity prevail but undoubtedly depends upon a number of intrinsic factors which involve the structure and properties of the cell envelope However the test constitutes a useful gross index of the relative thickness of the major number of red cells in the sample of blood to be tested If the red cell envelope is small in relation to its volume as in the thick red cells or spherocytes of hereditary spherocytosis the resistance is decreased Increased resistance to hemolysis is noted when the bulk of red blood cells possess relatively larger surfaces in comparison with their substance as occurs in the thin red cells of patients with Mediterranean anemia and in patients with iron deficiency anemia Sickle cells are also more resistant to *hypotonic saline solution than are normal cells*

Studies of the fragility of red blood cells of the newborn infant led to equivocal results varying from decreased to increased resistance In our laboratory mod erately increased resistance has been observed in the cord blood of the full term infant but is more marked in the premature infant On the other hand a slightly increased or normal fragility has been found[1] in the cord blood of the normal newborn infant In these studies it was noted that the erythrocytes of most chil dren with congenital cyanotic heart disease had only slightly greater fragility than those of normal patients These purely in vitro studies bear no relation to the shortened life span of fetal red blood cells when they are tagged with Cr^{51} and injected into normal individuals

Mechanical Fragility Tests on mechanical fragility which evaluate the strength of the red cell membrane by exposing erythrocytes to the trauma of rolling glass beads under prescribed conditions are regarded as more closely simulating physio logic conditions prevailing in the circulation With this test it was found[1] that

erythrocytes of the newborn infant break down more rapidly in the first days of life This increased mechanical fragility which falls to normal by the fifth and sixth days was associated with a rise in serum bilirubin Although red cells and hemoglobin levels show minimal quantitative fluctuations in the first days of life it is possible that shifts in plasma volume may obscure the rapid elimination of microcytic cells existing during this period According to this concept physiologic jaundice stems from at least two sources a hemolytic component and hepatic immaturity However current emphasis has been placed on the latter factor as being of major importance

Red Cell Thickness and Oxygen Dissociation It has been demonstrated by animal experiments that thickness of the red cells bears a relation to oxygen dissociation Thus the blood of patients with spherocytic cells is less able to deliver oxygen to the tissues than is blood of normal patients or of patients with anemias in which the red cells are reduced in thickness[47]

Erythrocyte Production Multiple factors many of which have specialized functions enter into the production of red cells

Factors Required for Red Cell Production Normal maturation of erythrocyte precursors requires amino acids protein and the B vitamins especially pyridoxine folic acid (pteroylglutamic acid) riboflavin vitamin B_{12} and the nucleic acid constituents thymine and thymidine Of these factors vitamin B_{12} folic acid and ascorbic acid are fundamentally involved in the development of the red blood cell Folic acid participates in red cell maturation only after conversion largely by ascorbic acid to its biologically active form folinic acid or citrovorum factor Vitamin B_{12} constitutes both the extrinsic factor and the erythrocyte maturation factor The intrinsic factor from the stomach is necessary for the absorption of vitamin B_{12} Whereas patients with pernicious anemia respond to either folic acid or vitamin B_{12} those with megaloblastic anemia of infancy in whom similar abnormal cells appear in the bone marrow usually respond to folic acid alone but rarely to vitamin B_{12} Both folinic acid (or folic acid) and vitamin B_{12} serve as coenzymes in nucleic acid synthesis in the production of thymine and thymidine

Factors required in red cell (stroma) formation and hemoglobin (heme and globin) synthesis overlap to the extent that the separate consideration of each process is not always feasible Although the need for minerals applies more particularly to the elaboration of hemoglobin many of the vitamins and amino acids are involved in both processes For example a deficiency in minerals results in both lowered hemoglobin levels and morphologic changes in the red blood cell Many of the inherited abnormal hemoglobins are associated with the presence of such definitive changes in the erythrocytes as sickle cells and target cells To overcome the need for a sharp division and to allow for this interrelationship the term erythropoiesis is therefore frequently employed to cover both processes

Humoral Regulation of Erythropoiesis (Hemopoietin or Erythropoietin) The physiologic balance between red cell production and destruction which results in a remarkable consistency of number and volume involves the participation of several regulatory mechanisms These are manifested in the stimulation of erythro

poiesis after hemorrhage and after exposure to an atmosphere of reduced oxygen tension

Bone marrow anoxia provides the primary stimulus for erythropoiesis as evidenced by polycythemia in chronic hypoxic states and the suppression of erythropoiesis when oxygen concentration is increased It is not yet clear whether anoxia acts directly on the bone marrow or on the organism to produce a stimulating impulse It is probable that lowered oxygen tensions stimulate bone marrow activity by a mediation of a humoral factor hemopoietin or erythropoietin

The ability of the serum and plasma of bled animals to induce a rise of reticulocytes red blood cells and hemoglobin when injected into normal animals gives further support to the presence of such a humoral factor The existence of this factor was originally postulated in 1906 by Carnot and Deflandre who transfused plasma from a bled animal into an intact animal Reismann [8] presented the first convincing evidence for the existence of a humoral factor by demonstrating similar hyperplasia of the bone marrow in a pair of parabiotic rats following chronic hypoxia induced in one partner Erslev and Lavietes[1, 16] stimulated further interest in this mechanism by the bioassay of large amounts of plasma from bled rabbits and monkeys which had induced reticulocytosis and other evidences of *increased erythropoietic activity of the bone marrow in normal* animals

In the human subject data have become available compatible with the humoral regulation of erythropoiesis Plasma filtrates from patients with severe Cooley's anemia were found to stimulate hematopoiesis in intact rats whereas similar preparations from normal subjects and one patient with chronic hypoplastic anemia were inactive [37] Gurney and associates reported finding increased erythropoietin (erythrocyte stimulating factor activity) in the serum of two patients with congenital hypoplastic anemia

The suppression of erythropoiesis after hypertransfusion[5, 13] may indicate the mediation of humoral regulation in response to a lessened need for erythropoiesis On the other hand the similar depression of hematopoiesis following multiple transfusions[44] in chronically anemic patients suggests regulation in different ways or degrees from those involved in the normal individual It appears possible that the physiologic adjustments in the chronically anemic person are such that hemoglobin *globin concentrations which would be considered* indicative of severe anemia under ordinary circumstances are in fact "normal for that patient

The effect of cobalt in increasing the rate of erythropoiesis in man and experimental animals to produce polycythemia has been attributed to bone marrow anoxia as a consequence of a toxic action on one or more enzyme systems It has been shown that certain of the properties of erythropoietin in plasma of anemic patients are also common to those of the active factor in cobalt plasma indicating that cobalt enhances red cell production by increasing the formation of erythropoietin

The actual site where the erythrocyte stimulating factor is produced has not yet been clearly determined Emphasis has been placed on the kidney is being directly or indirectly concerned with formation of the factor rather than the hemopoietic or other tissues [3, 7]

Erythropoietin is a protein found in highest concentrations in patients with diseases associated with erythroid aplasia.[4] Significant amounts have been noted in congenital hypoplastic anemia, aplastic anemia, acute lymphoblastic leukemia and chloramphenicol induced bone marrow aplasia. In patients with hemolytic conditions associated with erythroid hyperplasia as Cooley's anemia and sickle cell disease significant levels of erythropoietin are found; however, they are found less frequently than in patients with the aplastic group of anemias. Low concentrations are observed in patients with uremia and chronic inflammation. The only sources of erythropoietin are the plasma and urine, but therapeutic use is limited until richer exogenous supplies become available. Although cobalt increases endogenous erythropoietin production, its use is handicapped by toxicity.[4]

Characteristics of Primitive Blood Cells. Primitive cells of both the red and white cell series possess similar structural characteristics and in their maturation reveal many points in common. The early blast forms are large, the cytoplasm is deeply basophilic, the nucleus occupies more space and stains less deeply than the cytoplasm (leptochromatic), the chromatin is finely granular, and one or more nucleoli are present. At this stage classification is facilitated by comparison with more mature cells with which they are associated by noting morphologic similarities. Maturation is accompanied by the following features: the primitive cell in each series becomes progressively smaller in the content of cytoplasm and nucleus, the basophilia of the cytoplasm lessens, the chromatin becomes more condensed, its original purplish color changes to dark blue, and the nucleoli disappear early. In the red cell the basophilia is replaced by hemoglobin. In the mature granulocyte the cytoplasm becomes faintly pink with specific granulation, in the lymphocyte a hyaline or sky blue color, and in the monocyte a gray blue with fine reddish blue granules.

Stages in Maturation of Red Blood Cells—General Principles. The normal progression of red cell maturation is based upon intracellular chemical changes. A knowledge of these changes aids in identification of individual cells.

NUCLEIC ACID AND CELLULAR DEVELOPMENT. Cellular growth and multiplication of cells are closely identified with the content of the nucleic acids, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Although they both occur together, the former predominates in the cytoplasm (especially in the mitochondria) and nucleolus and the latter in the chromatin of the cell nucleus. Deoxyribonucleic acid, present in the nuclear material of all living cells, is generally believed to be the principal component of genes. Rapid growth of all cellular types, especially during mitosis, is accompanied by increased concentrations of the nucleic acids.[4]

BASOPHILIA. Deep basophilia of the cytoplasm characterizes the stem cell and red and white cell precursors at the "blast" level of immaturity. The high content of ribose nucleic acid parallels the maximal cytoplasmic basophilia. The basophilic staining is ascribed to the affinity of nucleic acid in cytoplasm for the basic component (methylene blue) of Wright's stain or other polychromatic stains. In all cell types basophilia recedes with increasing maturity, and in the red cell its gradual replacement by hemoglobin results in an increased affinity for acid dyes (eosin).

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pernicious anemia and related megaloblastic anemia in five to seven days and following iron therapy in patients with iron-deficiency anemia in five to ten days

Abnormal Maturation (Deficiency of the Hematopoietic Factors—Liver Folic Acid and Vitamin B_{12}) The cells are abnormally large and the nucleus matures more slowly than cytoplasm

Promegaloblasts (erythrogones)
Regarded by some as common progenitors of both pathologic megaloblasts and of normoblasts may be regarded as earlier form of megaloblast large cell Cytoplasm abundant basophilic Nucleus light purplish staining chromatin open reticular pattern stippled no clumping as in pronormoblast several nucleoli present

Megaloblast (basophilic megaloblast)
Corresponds to promegaloblast Cytoplasm more intense dark blue basophilia Nucleus no nucleoli chromatin finely divided

Polychromatic Megaloblast (intermediate megaloblast)
Large cell Cytoplasm hemoglobin now present and basophilia decreased resulting in multicolored polychromatophilic cytoplasm Nucleus purplish still finely granular with occasional clumping

Orthochromatic Megaloblast (late megaloblast)
Larger than corresponding late normoblast Cytoplasm deeply eosinophilic Nucleus purplish blue reduced in size irregular clumping but reticular appearance still present never cartwheel shaped

Macrocyte
Larger than normal erythrocyte which it resembles measures 9 microns or more in diameter

The megaloblast series in contrast to corresponding normoblasts shows less clumping and greater retention of the fine reticulated appearance of the nucleus during maturation and at each stage is larger than the corresponding normoblast Cells with partial deficiency of the hematopoietic factor intermediate in nuclear structure between the megaloblast and normoblast appear in the bone marrow With increased deficiency megaloblasts appear whereas specific treatment (such as folic acid in infantile megaloblastic anemia) results in differentiation into normoblasts

Reisner[20] points out that the nuclear chromatin particles are more homogeneous in the normoblastic cell as compared with the diffuse particulate meshlike chromatin of the megaloblastic cells and that the ratio of mitotabl (that is capable of mitosis) to maturing cells is much increased in the latter Megaloblasts are conceived as red cell precursors with a prolonged resting phase between mitoses allowing a longer time for the dispersion of the chromatin throughout the nucleus The conditions most conducive to megaloblastic blood formation are states in which vitamin B_{12} or folic acid is deficient Normoblastic hematopoiesis results in normal erythrocytes as compared with the oval macrocytes of megaloblastic hematopoiesis Megaloblasts are found in the bone marrow not only in pernicious anemia and megaloblastic anemia of infancy and of intestinal origin but also in a number of miscellaneous states in which for various reasons the patient has levels of vitamin B_{12} and folic acid inadequate for the increased demands for hematopoiesis These include multiple myeloma hemolytic anemia idiopathic aplastic anemia chronic blood loss with superimposed acute hemorrhage myelofibrosis erythremic myelosis advanced cirrhosis of the liver after anticonvulsant drugs (phenytoin sodium [Dilantin][10] and primidone [Mysoline] and leukemia in which antifols and 6-mercaptopurine have been used)[20]

POLYCHROMASIA AND STIPPLING Admixtures of basophilic substance and hemoglobin in intermediate stages produce *polychromatophilia of the cytoplasm* Stippling (punctate basophilia) refers to the fine or coarse bluish violet granules found in the red cells Stippled cells are noted in a variety of clinical conditions such as lead poisoning and hemolytic and deficiency anemias of varying severity

ERYTHROPOIESIS Two types of red cell maturation can be described the pathologic form associated with a deficiency of hematopoietic or liver principle megaloblastic erythropoiesis and the normal process normoblastic erythropoiesis The recognition of the various stages in either normal or abnormal maturation depends upon cell size staining of the cytoplasm and nuclear conformation according to the principles already stated Only those cell types need be included here which are commonly observed in pediatric practice and can be identified by bone marrow aspiration The difficulties in distinguishing between megaloblasts and early cells of the normoblastic series are confined to two conditions in this age period megaloblastic anemia of infancy and the rare juvenile pernicious anemia Other forms of anemia involve the orderly maturation of the normoblastic series The term erythroblast has assumed a comprehensive connotation and includes all nucleated red cells both normal and pathologic

Normal Maturation It will be noted that in the normal maturation the cytoplasm and nucleus mature simultaneously and synchronously The stages of normal red cell development and their chief morphologic and staining characteristics may be outlined as follows

Pronormoblast (procrythroblast)

About twice the size of a normal mature erythrocyte Cytoplasm narrow rimmed deeply basophilic Nucleus light purplish vesicular granular slight clumping one or more nucleoli present

Basophilic normoblast (early normoblast or early erythroblast)

Smaller than pronormoblast Cytoplasm basophilic Nucleus darker purplish staining chromatin clumping more marked may be cart wheel nucleoli absent

Polychromatic normoblast (intermediate normoblast late erythroblast)

Cytoplasm less basophilic traces of hemoglobin present Nucleus shrunk more mature chromatin bluish black coarse clumped a light area resembling hemoglobin often seen at one pole of the nucleus

Orthochromatic normoblast (late normoblast)

Size somewhat larger than erythroblast Cytoplasm almost completely filled with hemoglobin late forms red staining eosinophilic Nucleus chromatin condensed dark homogenous structureless mass referred to as pyknotic

Reticulocytes

Slightly larger than normal erythrocyte Nucleus has been extruded Reticulum or filamentous substance varying in amounts and arrangements stains deep blue with brilliant cresyl blue the smallest amounts seen in those nearly mature Reticulum corresponds to basophilia of cytoplasm and is not related to nuclear remnants *mitochondria or hemoglobin In two to five days perhaps in less time* reticulocytes mature in circulation and normally number 0 5 to 1 5 per cent Reticulocytes reflect reactivity of the bone marrow an increase indicates accelerated hematopoiesis Reticulocytes show maximal peaks following specific treatment of patients with

tissues The major physiologic method by which red cells are destroyed is by fragmentation caused by the traumatic effects of circulation Erythrophagocytosis within the spleen and lytic factors present in the plasma and tissues are contributory agents in this physiologic process Phagocytosis of intact red cells may be observed occasionally in the peripheral blood and bone marrow in patients with erythroblastosis and acute leukemia

Although phagocytosis and hemolysis may be involved in this process these factors exert their influence largely in pathologic conditions Fragmented cells (schistocytes) formed in this manner also result from the wearing out of enzyme systems controlling the integrity of the cell or its membrane Stagnation in the spleen can also contribute to red cell dissolution by altering the spheroidicity and osmotic fragility

The destruction of the red blood cell is accompanied by a breakdown of hemoglobin Following preliminary oxidative opening of the protoporphyrin ring and the formation of a green bile pigment (iron protein complex) verdohemoglobin iron is split off Iron attached to a beta 1 globulin of the plasma (siderophylin transferrin) is transported in the blood stream to the bone marrow for the regeneration of hemoglobin or to the liver spleen and other organs where it is deposited in the form of ferritin and when excessive hemosiderin

Formation of Bilirubin Bilirubin is derived from hemoglobin It is the globin and iron free fraction of the hemoglobin molecule An increased destruction of red cells during hemolysis results in a corresponding increase in bile pigment production When iron is split off the compound has been stated to exist for a time as a bilirubin globin complex. Following the loss of iron the green bile pigment is formed The separation of globin and the degradation to bilirubin probably occur in the reticuloendothelial system Globin enters the body protein pool as amino acids to be utilized in hemoglobin formation

Bilirubin pigment is formed in the reticuloendothelial elements of the spleen, lymph nodes bone marrow liver and connective tissue (probably with the bone marrow as the most significant site) About 10 to 15 per cent of total bile pigment is derived from precursors other than hemoglobin[31,33] such as heme not used in the synthesis of hemoglobin myoglobins peroxidase catalase and cytochromes Bile pigments in human serum range from 0.5 to 0.8 mg per 100 ml. of blood and those in the serum of patients with chronic hemolytic anemias range from 1 to 3 mg rarely above 5 mg When normal bilirubin levels occur in a patient with hemolytic anemia it is due to the ability of a healthy liver to excrete excess quantities of the pigment The accumulation of indirect bilirubin in the plasma as occurs in patients with erythroblastosis and in those in a hemolytic crisis results from overloading of the liver with the products of blood destruction

London[3] includes the following considerations in evaluating the gross bile pigment metabolism the amount of hemoglobin which is being destroyed daily the functional capacity of the liver to excrete bilirubin into the bile the patency of the biliary tree the functional capacity of intestinal bacteria to reduce bilirubin to the urobilinogens the functional capacity of the liver to re-excrete urobilinogen and the threshold of the kidney for the excretion of direct bilirubin

The multiple designations given to the same blood cells have led to an attempt to standardize the nomenclature and to provide more precise criteria for identity of each cell type[8] The recommended terms for the red cell series and a few of their alternates include the following rubriblast (megaloblast hemocytoblast) *prorubricyte (pronormoblast) rubricyte (basophilic and polychromatic normoblasts)* and metarubricyte (orthochromatic normoblast) "Pernicious anemia type" is applied as a qualifying phrase to any cell in this series in which the morphologic changes are those of pernicious anemia

Normal Destruction of Erythrocytes The destruction of the red cell is accompanied by a number of phenomena of which those relative to the excretion of bile pigment are among the most important

Life Span of the Red Blood Cell The life span of the human red cell in the circulation is approximately 120 days corresponding to a normal rate of replacement of 0 83 per cent per day The methods used for this determination are based on the Ashby method of differential agglutination" in which the number of transfused group O red cells is determined periodically in the blood of the recipient of another group The same objective can be achieved with the use of compatible blood for the major blood groups but with differences in the M and N factors In either case the agglutination of the recipient s blood by appropriate sera permits the counting of the residual unagglutinated cells of the donor Comparable results for the normal life span have been obtained with the use of red cells tagged with an isotope of nitrogen (N^{15}) of iron (Fe^{59}) or of chromium (Cr^{51})

Radioactive sodium chromate can be satisfactorily employed to measure the circulating red cell mass blood volume and red cell survival and to localize the site of red cell destruction[28, 29] The method of labeling red cells by radioactive chromium has replaced the other more elaborate methods It affords a simple method for studying red cell survival in normal subjects and in patients with various hematologic disturbances By reinjecting blood which had been mixed with the sodium radiochromate into the circulation of the patient the longevity of the cells is measured in their natural environment The chromium taken into the cell becomes firmly attached to the globin portion of the hemoglobin molecule

The half life survival of Cr^{51} tagged red cells has been found to average from 26 ± 2 days[1] to 33 1 ± 3 2 days[40] When correction is made for the loss of radioactivity by elution from the red cell of 1 per cent per day the half life of chromated cells corresponds to the half life of transfused cells as determined by other methods A considerably reduced half life has been noted in many of the intrinsic hemolytic anemias or in blood dyscrasias accompanied by a hemolytic component[43]

Fetal red blood cells obtained from the umbilical cord and tagged with Cr^{51} are noted to have a shortened life span with a half life of 14 to 22 days when they are injected into normal individuals[6]

Normal Hemolysis With a normal life span of 120 days approximately 1 per cent of the red cells leave the circulation daily Under normal conditions worn out red cells are removed from the circulation by *cells of the reticuloendothelial* system in the spleen bone marrow and liver and to a lesser extent in other

filtered by the kidneys and that relatively insoluble indirect bilirubin is incapable of being filtered. Hence the indirect type of bilirubin does not appear in the urine despite excessive amounts in the blood. Increased excretion of bile by the liver, however, accounts for the excess of urobilinogen in the urine of patients with hemolytic anemia. On the other hand, the soluble direct bilirubin can be excreted by the kidney when it accumulates in the blood as it does in patients with obstructive jaundice and hepatic or duct disease.

Urobilinogen Excretion—an Index of Hemolysis. Bile pigments are derived from the breakdown of heme, and the amount found is related almost quantitatively to the hemoglobin produced and destroyed each day. The porphyrin portion of hemoglobin, exclusive of the iron, constitutes 3.5 per cent by weight of the hemoglobin molecule. Theoretically, all of the porphyrin of the destroyed hemoglobin is converted to bilirubin. Thus 1 gm. of hemoglobin yields 35 mg. of bilirubin.[8] However, 10 to 15 per cent more than the estimated quantity actually appears each day as urobilinogen.[7, 33] The precursors of this additional fraction are some porphyrin which is synthesized in the liver and not incorporated into red cells and porphyrin derived from the destruction of hemoproteins other than hemoglobin (myoglobin and cytochromes).

Bilirubin is excreted into the bile and passes into the colon, where it is reduced by bacterial action to a group of urobilinogens consisting of mesobilirubinogen and stercobilinogen. Oxidation in the urine and stool results in conversion of colorless urobilinogen to the colored urobilin. A portion of the urobilinogens is excreted in the stools but in large part is reabsorbed from the intestine to re-enter the liver in the portal circulation and is then re-excreted in the bile. A few milligrams leave the general circulation to be excreted in the urine.

Quantitative estimates of urobilinogen excretion are an index of hemoglobin breakdown since the bulk of bile pigments stem from this source. It has been estimated that 1 mg. of urobilinogen is derived from approximately 24 mg. of destroyed hemoglobin. Increased hemoglobin destruction is invariably associated with excessive urobilinogen excretion in the feces and usually in the urine. The presence or absence of urobilinogen in the urine largely depends upon the capacity of the liver for re-excretion in the bile. With hepatic damage or dysfunction from overload, re-excretion of urobilinogen is impaired, a spill-over in the blood occurs, and urobilinogen appears in the urine. The normal daily adult excretion of urobilinogen in the feces is 40 to 280 mg.;[4] in the urine, it is 0.5 to 3.5 gm. In infants and children the normal daily mean value for fecal urobilinogen ranges from 3.8 mg. under 1 year of age to 45.2 mg. from 10 to 14 years of age; in patients with hemolytic disease, it ranges from 15.1 mg. to 205 mg., respectively.[3]

Compensated Hemolytic Disease. The processes of blood destruction and formation are normally balanced so that the concentration of hemoglobin and red cells in the circulating blood remains unaltered. In a state of balance the red cells and hemoglobin destroyed are replaced each day. When blood destruction is increased, bone marrow activity is accelerated, more red cells and hemoglobin are produced, and equilibrium is re-established. Within limitations an excessive degree of hemolysis will not result in anemia in a patient with ade

Direct and Indirect Bilirubin. Two types of bilirubin have been differentiated on the basis of the van den Bergh reaction. The basis of this test is the observation by Ehrlich that the addition of diazo reagent (diazotized sulfanilic acid) to blood plasma or other bile-containing solutions yields a reddish violet color. The immediate evolution of maximum color intensity is termed a direct reaction. If *color fails to develop in one minute but appears after the addition of alcohol* the reaction is regarded as indirect. No completely satisfactory explanation has been offered for the direct and indirect reactions, although it is now known that the direct reaction depends upon the formation of soluble glucuronides of bilirubin.

The van den Bergh test employs these reactions to distinguish the two types of bilirubin involved in clinical jaundice. If the jaundice results from intrahepatic or extrahepatic obstruction to the passage of bile into the intestine, most of the bilirubin is of the direct reacting form. This is the one-minute bilirubin. When jaundice is due to excessive destruction of hemoglobin, as in hemolytic anemia, most of the bilirubin is in the indirect reacting form, that is, the addition of alcohol is essential for color to develop. It is possible that the alcohol catalyzes the transformation of indirect to direct bilirubin so that the diazo reagent will produce the reddish violet color. The total concentration of both types of bilirubin usually ranges from 0.2 to 1.4 mg per 100 ml, with 0.2 mg as the upper limit of normal for the direct type.

Klatskin and Bungards have shown that the *bulk of bilirubin in serum is bound to albumin* irrespective of its behavior in the van den Bergh reaction. They found no evidence that in the breakdown of hemoglobin globin remains attached to its porphyrin fraction to yield indirect reacting bilirubin. The action of alcohol in facilitating the diazotization of indirect reacting bilirubin does not depend upon release of bilirubin from its protein complex. This contradicts the former concept that the indirect reacting bilirubin is attached to alpha globulin in contrast to the linkage of direct reacting bilirubin to serum albumin.

It has also been postulated[38] that the direct bilirubin is a bilirubin metal complex and that indirect bilirubin represents bilirubin not bound to metal.

The excretion of bile depends upon the conversion of indirect to direct bilirubin. This process has heretofore been regarded as a function of enzyme systems in the liver which are active in the dissociation of pigment protein complexes. It has recently been *demonstrated*, however, *that the water insoluble indirect bilirubin requires conjugation with glucuronic acid for the formation of water soluble direct bilirubin* which is then capable of excretion in the bile. The terms bilirubin and conjugated bilirubin have therefore been suggested as substitutes for "indirect bilirubin" and "direct bilirubin," respectively.[4] According to this concept, indirect bilirubin, being water insoluble, requires prior addition of alcohol to initiate coupling with the diazo reagent, whereas conjugated bilirubin, being water soluble, is readily diazotized. The formation of bilirubin glucuronide is mediated by a glucuronyl transferase enzyme system and the delay in its formation accounts for the accumulation of indirect bilirubin in the plasma in jaundice of the newborn infant (see Chapter 8). In obstructive jaundice conjugated (direct) bilirubin gains access to the blood and then to urine.[3,41] It is thus understandable that soluble bilirubin glucuronides (direct reacting bilirubin) can be readily

filtered by the kidneys and that relatively insoluble indirect bilirubin is incapable of being filtered Hence the indirect type of bilirubin does not appear in the urine despite excessive amounts in the blood Increased excretion of bile by the liver however accounts for the excess of urobilinogen in the urine of patients with hemolytic anemia On the other hand the soluble direct bilirubin can be excreted by the kidney when it accumulates in the blood as it does in patients with obstructive jaundice and hepatic or duct disease

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Bilirubin is excreted into the bile and passes into the colon where it is reduced by bacterial action to a group of urobilinogens consisting of mesobilirubinogen and stercobilinogen Oxidation in the urine and stool results in conversion of colorless urobilinogen to the colored urobilin A portion of the urobilinogens is excreted in the stools but in large part is reabsorbed from the intestine to re enter the liver in the portal circulation and is then re excreted in the bile A few milligrams leave the general circulation to be excreted in the urine

Quantitative estimates of urobilinogen excretion are an index of hemoglobin breakdown since the bulk of bile pigments stem from this source It has been estimated that 1 mg of urobilinogen is derived from approximately 24 mg of destroyed hemoglobin Increased hemoglobin destruction is invariably associated with excessive urobilinogen excretion in the feces and usually in the urine The presence or absence of urobilinogen in the urine largely depends upon the capacity of the liver for re excretion in the bile With hepatic damage or dysfunction from overload re excretion of urobilinogen is impaired a spill over in the blood occurs and urobilinogen appears in the urine The normal daily adult excretion of urobilinogen in the feces is 40 to 280 mg[4] in the urine it is 0 5 to 3.5 gm In infants and children the normal daily mean value for fecal urobilinogen ranges from 3 8 mg under 1 year of age to 45 2 mg from 10 to 14 years of age in patients with hemolytic disease it ranges from 15 1 mg to 205 mg respectively[3]

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quately compensating bone marrow activity According to Crosby and Akeroyd[10] hemolytic anemia follows only when the average life span of the red blood cell (normally 120 days) becomes so short that the bone marrow working at maximum capacity (6 to 8 times the normal) cannot maintain an adequate output of hemoglobin and red blood cells They have emphasized the important fact that abnormal hemolysis without anemia is indicative of a completely compensated hemolytic process and that the underlying feature of the hemolytic syndrome is reduced survival of the red cells When these limits are exceeded and anemia develops equilibrium may be re established at lower levels depending upon the capacity of the bone marrow to form red cells and the extent of their survival in the circulation

When the rate of red cell destruction exceeds the rate of red cell production the red cell volume is lowered and fewer red cells are being destroyed per unit of time When the red cell volume has been reduced to that point at which the number of red cells being destroyed is equaled by the number of red cells being produced a new equilibrium is established resulting in a stable but anemic hemoglobin level[14] At any one time in a hemolytic disease a particular hemoglobin value is the resultant of the extent of the hemolytic defect present and the particular capacity of the bone marrow to respond with an increase in hemoglobin synthesis and red cell production

The concept of compensated hemolytic disease is important in appraising blood conditions in which blood levels appear unduly high in the face of a known hemolytic process Because of this mechanism anemia in hereditary spherocytosis (familial hemolytic anemia) may not always be present and in patients with the trait or mild form of Mediterranean anemia overcompensation accounts for the polycythemia at times in association with elevated hemoglobin levels Evidences of increased hemolysis can be detected with the aid of appropriate laboratory tests A significant increase of reticulocytes represents a simple index of compensated hemolytic disease In erythroblastosis fetalis augmented reticulocyte values with a normal red cell count on the first day of life may indicate increased red cell turnover and the need for close watch in succeeding days for the development of anemia and jaundice In crises of sickle cell anemia the hemoglobin level *often remains unchanged but increased reticulocytes reflect compensatory* bone marrow activity

REFERENCES

1 Adams F H and Cunningham S C Fragility of Red Blood Cells From Newborn Infants and Children With Cyanotic Congenital Heart Disease J Pediat 39 180 1951

2 Ashby W Determination of Length of Life of Transfused Blood Corpuscles in Man J Exper Med 29 267 1919

3 Billing B H and Lathe G H The Excretion of Bilirubin as an Ester Glucuronide Giving the Direct van den Bergh Reaction Biochem J 63 6 1956

4 Billing B H and Lathe G H Bilirubin Metabolism in Jaundice Am J Med *24 111* 1958

5 Birkhill F R Maloney M A and Levenson S M Effect of Transfusion Polycythemia Upon Bone Marrow Activity and Erythrocyte Survival in Man Blood 6 1021 1951

6 Bolande R I Traisman H S and Philipsborn H F Jr Electrolyte Considerations in Exchange Transfusions for Erythroblastosis Fetalis *J Pediat 49 401 1956*

7 Carnot P and Deflandre C Sur l'activité hémopoiétique du sérum Compt rend Acad sc 143 384 1906
8 Condensation of the First Two Reports of the Committee for Clarification of the Nomenclature of Cell and Diseases of the Blood and Blood Forming Organs Blood 4 89 1949
9 Crosby W H The Hemolytic States Bull New York Acad Med 30 27 1954
10 Crosby W H and Akeroyd J H The Limit of Hemoglobin Synthesis in Hereditary Hemolytic Anemia Am J Med 13 273 1952
11 Donohue D M Finch C A and Gabrio B W Erythrocyte Preservation the Storage of Blood With Purine Nucleosides J Clin Invest 35 562 1956
12 Donohue D M Motolsky A G Giblett E R Pirsio Biroli G Viranuvatti V and Finch C A The Use of Chromium as a Red Cell Tag Brit J Haemat. 1 249 1955
13 Emlinger P J Huff R L and Oda J M Depression of Red Cell Iron Turnover by Transfusion Proc Soc Exper Biol & Med 79 16 1952
14 Erlandson M E Schulman I Stern G and Smith C H Studies of Congenital Hemolytic Syndromes Rates of Destruction and Production of Erythrocytes in Thalassemia, Pediatrics 22 910 1958
15 Erslev A Humoral Regulation of Red Cell Production Blood 8 349 1953
16 Erslev A and Lavietes P H Observations on the Nature of the Erythropoietic Serum Factor Blood 9 1055 1954
17 Gabrio B W Donohue D M and Finch, C A Erythrocyte Preservation Relationship Between Chemical Changes and Viability of Stored Blood Treated With Adenosine J Clin Invest 34 1509 1955
18 Gabrio B W Finch C A and Huennekens F M Erythrocyte Preservation a Topic in Molecular Biochemistry Blood 11 103 1956
19 Girdwood R H and Lenman J A R Megaloblastic Anaemia Occurring During Primidone Therapy Brit M J 1 146 1956
20 Goldbloom A and Gottlieb R Icterus Neonatorum Am J Dis Child 38 57 1929
21 Goldbloom R B Fischer E Reinhold J and Hsia D Y Y Studies on the Mechanical Fragility of Erythrocytes Normal Values for Infants and Children, Blood 8 165 1953
22 Goldwasser E Jacobson L O Fried W and Plzak, L Mechanism of the Erythropoietic Effect of Cobalt Science 125 1085 1957
23 Gordon A S Hemopoietine Physiol Rev 39 1 1959
24 Granick, S The Chemistry and Functioning of the Mammalian Erythrocyte Blood 4 404 1949
25 Grant, W C and Root, W S Fundamental Stimulus for Erythropoiesis Physiol. Rev 4 449 1952
25a Gurney C W Pierce M I Schrier S E Carson P E and Jacobson, L O The Stimulatory Effect of "Anemic Plasma" in Congenital Hypoplastic Anemia J Lab & Clin Med 50 821 1957
26 Hollingsworth, J W Life Span of Fetal Erythrocytes J Lab & Clin. Med 45 469 1955
27 Jacobson, L O Goldwasser E Fried W and Plzak L Role of the Kidney in Erythropoiesis Nature 179 633 1957
28 Jandl, J H Greenberg M S Yonemoto R H and Castle W B Clinical Determination of the Sites of Red Cell Sequestration in Hemolytic Anemias J Clin Invest 35 842 1956
29 Jones N C Hughes and Szur L Determination of the Sites of Red-Cell Destruction Using CR Labelled Cells Brit J Haemat 3 320 1957
30 Jones P N Weinstein I M Ettinger R H and Capps R B Decreased Red Cell Survival Associated With Liver Disease Use of Radioactive Sodium Chromate in Measurement of Red Cell Survival A.M.A. Arch Int Med 95 93 1955
31 Klatskin G and Bungards L Bilirubin Protein Linkages in Serum and Their Relationship to the van den Bergh Reaction J Clin Invest 35 537 1956
32 London I M Metabolism of Hemoglobin and of Bile Pigment Bull Acad Med 30 509 1954

33 London I M West R Shemin D and Rittenberg D On the Origin of Bile Pigment in Normal Man J Biol Chem 184 351 1950
34 Miller G McCoord A B Joos H A and Clausen S U Studies of Serum Electrolyte Changes During Exchange Transfusion Pediatrics 13 412 1954
35 Mill S D and Mason H L Values for Fecal Urobilinogen in Childhood A M A Am J Dis Child 84 322 1952
36 Najjar V A The Metabolism of Bilirubin Pediatrics 10 1 1952
37 Piliero S J Medici P T Pansky B Luhby A L and Gordon A S Erythropoietic Stimulating Effects of Plasma Extracts From Anemic Human Subjects Proc Soc Exper Biol & Med 93 302 1956
38 Reissmann K R Studies on the Mechanism of Erythropoietic Stimulation in Parabiotic Rats During Hypoxia Blood 5 372 1950
39 Reisner E H Jr The Nature and Significance of Megaloblastic Blood Formation Blood 13 313 1958
39a Rimington C Biosynthesis of Haemoglobin Brit M Bull 15 19 1959
40 Ryan C M S and Forshaw J W B Megaloblastic Anaemia Due to Phenytoin Sodium Brit M J 2 242 1955
41 Schmid R Direct Reacting Bilirubin Bilirubin Glucuronide in Serum Bile and Urine Science 124 76 1956
42 Schwartz H C Cartwright G E Smith E L and Wintrobe M M Studies on the Biosynthesis of Heme From Iron and Protoporphyrin Blood 14 486 1959
43 Smith C H Adsorption of Methylene Blue by Blood of Infants and Children Mechanism of Reaction and Clinical Application Am J Dis Child 57 1223 1939
44 Smith C H Schulman I Ando R E and Stern G Studies in Mediterranean (Cooley s) Anemia The Suppression of Hematopoiesis by Transfusions Blood 10 707 1955
45 Stohlman F Erythropoietine Pediatrics 23 835 1959
46 Tullis J L Blood Cells and Plasma Proteins Their State in Nature New York 1953 Academic Press Inc p 242
47 Valtis D J and Baikie A G The Influence of Red Cell Thickness on the Oxygen Dissociation Curve of Blood Brit J Haemat 1 152 1955
48 Watson C J Medical Progress Bile Pigment New England J Med 227 665 1942
49 Weinstein I M Spurling C L Klein H and Necheles T F Radioactive Sodium Chromate for the Study of Survival of Red Blood Cells III The Abnormal Hemoglobin Syndrome Blood 9 1155 1954
50 Young L E Hemolytic Disorders Some Highlights of Twenty Years of Progress Ann Int Med 49 1073 1958

CHAPTER 5

Erythrocytes—Morphologic Abnormalities

General Considerations Of all of the laboratory procedures the most important for its diagnostic value yet the simplest is the examination of the blood smear Although corroborative evidence from auxiliary sources may be required to establish a final diagnosis the stained film constitutes a visual representation of the effect on morphology of the factors involved in the pathogenesis of a specific anemia In describing the changes in the red blood cells it should be pointed out that there are few specific red cells that are indicative of a particular disorder Target cells oval cells hypochromic macrocytes and basophilic stippling and hypochromic microcytes appear in varying percentages in certain stages of many anemias and therefore cannot be regarded as distinguishing features of a single disease They are significant with the support of other pertinent information

Although overlapping occurs and differentiation from the normal is often difficult the following represent the most conspicuous alterations in size shape and structural changes and staining peculiarities of the erythrocytes

Abnormalities in Size Following are abnormalities of size which may be noted

Anisocytosis The excessive variation in size of the red cells (average diameter 7 5 microns) is termed anisocytosis

Poikilocytosis Marked irregularity in the shape of the red cell is termed poikilocytosis The pear shaped cell is most commonly found in those patients with anemias in which there is marked variation in size of the cells

Microcytes Microcytes are cells with diameters of less than 6 5 microns and usually characterize the blood smear of the patient with iron deficiency anemia Microblasts or microcytic nucleated red cells are increased in the bone marrow of patients with iron deficiency or blood loss Pointed projections may extend from the cytoplasm The chromatin of the nucleus is condensed and deeply stained

Macrocytes Macrocytes are found principally in patients in whom there is a deficiency of liver maturation principle such as in those with pernicious anemia or in patients with a deficiency of folic acid such as in megaloblastic anemia of infancy They are large cells with a diameter of 8 5 microns or more are thicker than the normocyte are well filled with hemoglobin and possess a mean corpuscular volume greater than normal

Hypochromic Macrocytes Hypochromic macrocytes are found in small num

bers in the blood of patients with many of the chronic anemias of childhood They appear in larger numbers in the blood of patients with Mediterranean anemia of varying grades of severity they are of especial value in the diagnosis of the mild form Normochromic *macrocytes are observed in small numbers in the blood* smear of the patient with aplastic anemia in childhood

Spherocytes (Microspherocytes) In hereditary spherocytosis the spherical shape of the cells results from a developmental defect which occurs after the erythroblast stage and in which a normal cell volume is enclosed within a greatly diminished *surface area Spherocytes are globular thick red cells of lessened diameter* but usually are normal in volume hence they are readily hemolyzed in hypotonic solutions of sodium chloride Since normal red cells become thicker as they take up water spherocytes already globular require less water before hemolysis sets in Mechanical fragility is also increased The small deeply stained spherocytes which characterize congenital spherocytic anemia may also be observed in varying degrees in patients with other conditions associated with hemolysis such as acquired hemolytic anemia and erythroblastosis due to sensitization by the A B agglutinogens and less frequently in patients with congenital nonspherocytic hemolytic anemia leukemia and conditions of stasis within the spleen and in stored blood Only the peculiar shape of these cells renders them susceptible to trapping by the spleen

Abnormalities in Shape Another morphologic abnormality of erythrocytes is irregularity of shape

Elliptocytosis (Ovalocytosis) Oval or elliptical cells occur typically as a dominant hereditary anomaly in which they constitute from 25 to 90 per cent of all erythrocytes The cells vary in shape from the rod or elongated forms to the oval shape all being well filled with hemoglobin Contrary to sickle cells these cells cannot be made to sickle in an environment of lowered oxygen tension Elliptocytosis is usually benign and symptomatic however asymptomatic mild compensated anemia and more rarely overt hemolytic anemia have been reported as complications in about 15 per cent of the patients[14]

The average survival of elliptocytes transfused into a normal recipient was found to be about 30 days[9] or 60 days by the Ashby technique[10] in contrast to the normal of 120 days Measured by sodium chromate (Cr^{51}) the survival of the patient s red cells within his own circulation is considerably reduced with a half life of 18 5 days as compared with the half life of normal cells of 33 1 days[16] Nucleated red cell precursors show no abnormalities of shape Elliptical cells occur in smaller numbers in the blood of patients with sickle cell disease[3, 9] and in that of the newborn infant[1] They do not reach their maximum level in the infant until he is 3 to 4 months of age On the other hand ovalocytosis may be associated with hemolytic anemia and be accompanied by hyperbilirubinemia soon after birth in such cases exchange transfusion should be considered

Oval and elliptical cells are often noted in association with marked anisocytosis and poikilocytosis It is necessary therefore to differentiate symptomatic from hereditary elliptocytosis[1] since oval shaped cells appear in variable numbers in the blood of patients with Mediterranean anemia severe iron deficiency anemia pernicious anemia anemia of infection leukemia and hereditary nonspherocytic

hemolytic disease Also included is a type of familial hypochromic microcytic anemia with splenomegaly affecting male members

Sickle Cells (Drepanocytes) The characteristic cells are elongated and narrow with rounded pointed or filamentous ends Although they appear in the stained smears of the severely anemic patient they are greatly increased in number in sealed wet films with reduced oxygen tension Reversion to the normal form occurs with exposure to oxygen or carbon monoxide Frequently in the trait or sicklemia the cells assume a holly leaf appearance exhibiting numerous superficial spines The mature orthochromatic normoblast can be made to sickle slowly although stained bone marrow smears show no morphologic changes in the nucleated red cells

The sickling phenomenon is based on the presence of an abnormal form of hemoglobin which can be separated from normal hemoglobin by differences in electrophoretic mobility [19] This disparity between the two hemoglobins is caused by the globin rather than the heme part of the molecule The peculiar shape of the erythrocytes has been attributed to the greatly diminished solubility of reduced sickle cell hemoglobin as compared with reduced normal hemoglobin This results in intracellular crystallization of the hemoglobin[19] and increased viscosity with tactoid formation resembling sickled erythrocytes [8] A minimal value of at least 20 per cent of sickle hemoglobin is required to elicit the sickling phenomenon

Ovalocytosis has been observed in association with the sickle cell trait with evidence of both varieties of cells in the peripheral blood [5]

Target Cells In target cells hemoglobin is concentrated in the periphery and in the center producing concentric light and dark zones after staining The deeply stained central area gives the impression of a bull's eye in a target [1] Target cells are a variety of leptocytes or thin cells whose cell envelope is too large and out of proportion to its meager contents and accounts for their increased osmotic resistance in hypotonic solutions of sodium chloride

Target cells originally regarded as diagnostic of Mediterranean anemia are now known to be nonspecific and are found in increased numbers in many anemic states in infants and children in patients with obstructive jaundice and hypochromic anemia following splenectomy and especially in patients with conditions associated with abnormal hemoglobins Target cells are present in patients with both mild and severe Mediterranean anemia (usually up to 10 per cent) are further increased in those with sickle cell anemia and appear in larger numbers in patients with a combination of these diseases (microdrepanocytic or thalassemia sickle cell disease) These cells are prominent also in the blood of patients with the hemolytic diseases associated with other pathologic hemoglobins In those with sickle cell hemoglobin C disease for instance they are present to the extent of 40 to 85 per cent of all red cells [17] It has been suggested that large numbers of target cells in the blood smear indicate the presence of C hemoglobin Whereas target cells are also increased in patients with hemoglobin E disease perhaps less than in those with C hemoglobin few or none of these cells has been noted in reported cases of sickle cell-hemoglobin D disease [7]

Leptocytes Leptocytes are abnormally thin erythrocytes some are bowl

shaped and assume the appearance of the target cell following drying and preparation of the film Leptocytosis refers to a preponderance of leptocytes such as occur in patients with hypochromic microcytic anemia and Mediterranean anemia The term hereditary leptocytosis is an alternative name for the latter disease Other cells found in the latter condition are nontarget cells but are extremely thin leaflike and of remarkable transparency

Miscellaneous Changes Miscellaneous morphologic abnormalities in erythrocytes are considered in the following discussions

Basophilic Stippling Basophilic stippling or punctate basophilia already described applies to the presence of round fine or coarse bluish violet or dark blue granules scattered in the cytoplasm of the polychromatophilic red blood cells They represent regeneration or immaturity of the cell and are sometimes best observed in slightly thicker and moderately overstained blood smears They are associated with all chronic anemias leukemia iron deficiency anemia the trait or mild form of Mediterranean anemia and lead poisoning They are to be differentiated from the network found in reticulocytes in cresyl blue preparations

Hypochromia and Hyperchromia Hypochromia is observed in cells with a lack of hemoglobin and is represented by an increase in central pallor Deeply and homogeneously stained red cells lacking central pallor because of complete hemoglobinizations are termed hyperchromic A macrocyte with a normal concentration of hemoglobin contains more of this substance than a normocyte only because of its size Since oversaturation with hemoglobin does not occur the cell cannot be truly regarded as hyperchromic Nevertheless according to common usage hyperchromic anemias identify conditions in which macrocytes prevail and in which the color index is greater than unity

Siderocytes Siderocytes are red blood cells containing nonhemoglobin iron particles which are visualized by the Prussian blue method of staining Sideroblasts are normoblasts containing similar iron inclusions Both types of cells in the peripheral blood and bone marrow respectively appear in moderate numbers in normal persons They are increased in those with infection aplastic and hemolytic anemias and are markedly decreased in those with iron deficiency anemias [1] Siderocytes are significantly increased after splenectomy

Heinz (Heinz Ehrlich) Bodies Heinz bodies are moderately sized round or irregularly shaped protein containing granules lying at or close to the periphery of the red blood cell They are single or multiple refractile inclusion bodies and are observed after supravital staining with brilliant cresyl blue and methyl violet The Romanowsky dyes such as Wright s stain obscure their presence They are easily detected as refractile bodies in unstained wet preparations These intracellular inclusions have been described as refractile agglomerations of denatured globin verdohemoglobin and perhaps stromal material Heinz bodies represent rather severe intoxication of the red cell[3] and are particularly noticeable in the presence of intrinsic defects within the cell They are to be differentiated from Howell Jolly bodies reticulocytes and siderotic granules

The presence of Heinz bodies in significant numbers is indicative of injury to the red cells and serves as an index of existing or impending anemia Sulfonamides primaquine phenylhydrazine naphthalene phenacetin and the fava

been associated with red cell destruction are prominent among Heinz forming compounds[6] They appear in increased numbers in patients with hemolytic anemias and leukemia following splenectomy [4] and in patients with agenesis of the spleen (See Chapters 8 and 15)

Howell Jolly Bodies Howell Jolly bodies are small rounded densely staining nuclear remnants occurring singly or doubly and are eccentrically placed in the red cell They stain a reddish blue or dark violet with Wright s stain and are prominent after splenectomy and in many anemias such as severe iron-deficiency anemia pernicious anemia spherocytic anemia and leukemia The origin of these bodies has been ascribed to abnormal mitosis in the late megaloblast stage when single chromosomes or groups of chromosomes become detached fail to be included in the formation of the interphase nucleus and remain free in the cytoplasm as nuclear remnants [11]

The presence of target cells siderocytes normoblasts and Howell Jolly and Heinz bodies in varying combinations in the peripheral blood of a young infant prompts consideration of agenesis of the spleen especially when associated with serious anomalies of the heart and situs inversus of the abdominal vicera [4] It has been stated that the diagnosis of asplenia can be made with confidence in infants with congenital cardiac disease who are polycythemic and whose peripheral blood shows numerous Howell Jolly bodies [1]

Cabot Rings Cabot rings are basophilic rings circular or twisted into a figure of eight which occur occasionally in red blood cells of patients with hemolytic anemias untreated pernicious anemia leukemia and lead poisoning They stain reddish purple with Wright s stain Originally regarded as nuclear remnants they are now interpreted as an expression of cellular degeneration resulting from the action of hemolytic agents [1]

Crenation In blood smears which dry slowly the red cell envelope becomes exposed to a hypertonic medium which causes wrinkling of the surface with the appearance of a moderate number of knoblike or prickly projections This process known as crenation occurs normally Two other types of crenated cells have been described which however are of pathologic significance

Burr Cells The burr cell is a mature red cell possessing one to several spiny projections along its periphery and represents a type of preformed poikilocyte Burr cells have been especially noted in persons with conditions with impaired renal function They appear in infants in connection with toxic hemolytic anemia associated with Heinz body function In the first trimester of life they appear in small numbers in normal full term and premature infants (approximately 2 to 6 per cent maximally respectively) and increase transiently up to 50 per cent in patients with severe hemolytic anemia [9] Burr cells may however appear sporadically beyond this age in patients with severe hemolytic disorders

Acanthrocytes Acanthrocytes (thorny red cells) resemble the burr cell but in the stained film look like spherocytes with pseudopods The projections are large coarse and irregularly spaced These malformed cells were observed in large numbers in the blood of a child with progressive ataxic neuropathy [6] The differentiation between acanthrocytes and burr cells may be difficult and bizarre

erythrocytes of an intermediate type have been observed as an inherited abnormality in association with retinal pigmentary degeneration

Familial Erythroid Multinuclearity A familial erythroid anomaly has been observed[30] in which giant sized erythrocytes giant plurinuclear red cell precursors and nucleated red cells with coarse cytoplasmic stippling and karyorrhexis have been found in the bone marrow Although persons with this anomaly have no symptoms they have a tendency toward mild anemia with peripheral blood showing anisocytosis with a tendency toward macrocytosis and poikilocytosis

REFERENCES

1 Barrett A M Special Form of Erythrocytes Possessing Increased Resistance to Hypotonic Saline J Path & Bact 46 603 1938

2 Bassen F A and Kornzweig A L Malformation of the Erythrocytes in a Case of Atypical Retinitis Pigmentosa Blood 5 381 1950

3 Bevan G H and White J C Oxidation of Phenylhydrazines in the Presence of Oxyhemoglobin and the Origin of Heinz Bodies in Erythrocytes Nature 173 389 1954

4 Bush J A and Ainger L E Congenital Absence of the Spleen With Congenital Heart Disease Pediatrics 15 93 1955

5 Fadem R S Ovalocytosis Associated With the Sickle Cell Trait Blood 4 505 1949

6 Fertman M H and Fertman M A Toxic Anemia and Heinz Bodies Medicine 34 131 1955

7 Gasser von C and Willi H Spontane innenkorperbildung dei milzagenesie Helvet paediat acta 7 369 1952

8 Harris J W Studies on the Destruction of Red Blood Cells Molecular Orientation in Sickle Cell Hemoglobin Solutions Proc Soc Exper Biol & Med 75 197 1950

9 Hedenstedt S Elliptocyte Transfusions as a Method in Studies on Blood Destruction Blood Volume and Peritoneal Resorption Acta chir scandinav (suppl 128) 95 1 1947

10 Hurley T H and Weisman R Jr The Determination of the Survival of Transfused Red Cells by a Method of Differential Hemolysis J Clin Invest 33 835 1954

11 *Hutchinson H E and Ferguson Smith M A The Significance of Howell Jolly Bodies* in Red Cell Precursors J Clin Path 12 451 1959

12 Kaplan E Zuelzer W W and Mouriquand C Sideroblasts a Study of Stainable Non hemoglobin Iron in Marrow Normoblasts Blood 9 203 1954

13 Kaplan E Zuelzer W W and Neil J V Further Studies on Hemoglobin C the Hematologic Effects of Hemoglobin C Alone and in Combination With Sickle Cell Hemoglobin *Blood* 8 735 1953

14 Lipton E L Elliptocytosis With Hemolytic Anemia the Effects of Splenectomy Pediatrics 15 67 1955

15 Lyons W S Hanlon D G Helmholz H F Jr and Edwards J E Congenital Cardiac Disease and Asplenia Report of Seven Cases Proc Staff Meet Mayo Clin 32 277 1957

16 McBryde R R Hewlett J S and Weisman R Jr Elliptocytosis a Study of Erythrocyte Survival *Using Radioactive Chromium* (CR^{51}) *Am J M Sc 232 258 1956*

17 Motulsky A V Singer K Crosby W H and Smith V The Life Span of the Elliptocyte Hereditary Elliptocytosis and Its Relationship to Other Familial Hemolytic Diseases Blood 9 57 1954

18 Pauling L Itano A H Singer S J and Wells I C Sickle Cell Anemia a Molecular Disease Science 110 543 1949

19 Perutz M F and Mitchison J M *State of Haemoglobin in Sickle Cell Anemia Nature* 166 677 1950

20 Rundles R W and Falls H F Hereditary (?Sex Linked) Anemia Am J M Sc 211 641 1946

21 Schleicher F M Origin and Nature of the Cabot Ring Bodies of Erythrocytes J Lab & Clin Med 27:983 1942
22 Schwartz S O and Motto S A The Diagnostic Significance of Burr Red Blood Cells Am J M Sc 218 563 1949
23 Scott R B Crawford R P and Jenkins M Incidence of Sicklemia in the Newborn Negro Infant Am J Dis Child 75 842 1948
24 Selwyn J G Heinz Bodies in Red Cells After Splenectomy and After Phenacetin Administration Brit J Haemat 1:173 1955
25 Singer K and Fisher B Studies on Abnormal Hemoglobins Blood 8 270 1953
26 Singer K Fisher B and Perlstein M A Acanthrocytosis a Genetic Erythrocytic Malformation Blood 7 577 1952
27 Sturgeon P Itano H A and Bergren W R Clinical Manifestations of Inherited Abnormal Hemoglobins the Interaction of Hemoglobin S With Hemoglobin D Blood 10 389 1955
28 Tuffy P Brown A K and Zuelzer W W Infantile Pyknocytosis a Common Erythrocyte Abnormality of the First Trimester Society for Pediatric Research Twenty Ninth Annual Meeting May 8 1959
29 Watson J Stahman A W and Bilello F P The Significance of the Paucity of Sickle Cells in Newborn Negro Infants Am J M Sc 215 419 1948
30 Wolff J A and von Hofe F H Familial Erythroid Multinuclearity Blood 6 1274 1951
31 Wyandt H Bancroft I M and Winship T O Elliptic Erythrocytes in Man Arch Int Med 68 1043 1941

CHAPTER 6

Blood Groups

Blood Group Factors Blood groups factors are genetically determined substances on the surfaces of erythrocytes which are present in certain persons and absent in others[7] The blood group factor is antigenic and is that portion of the red cell which combines with a specific antibody Of the blood systems the ABO and Rh groups are most important clinically

Naturally occurring antibodies in significant concentration are normally absent in the Rh group and numerous minor blood types and are present in the ABO groups Both the ABO and Rh factors are antigenic and if they are introduced into a host in whom they are absent antibody is formed Each factor is highly specific and can be differentiated by its behavior with the corresponding antibody If cells containing one of these antigens are suspended in serum containing the corresponding antibody the antibody unites with the antigen with resulting agglutination of the red cells which may then undergo lysis The significance of *the blood group factors stems from hemolytic reactions when red cells are trans*fused into recipients with corresponding antibodies within the circulation and during pregnancy when antibodies of maternal origin cross the placenta destroying fetal red cells Thus the blood group systems ABO and Rh are of paramount importance in the pathogenesis of hemolytic transfusion reactions and in erythroblastosis

Definitions of Terms in Relation to Blood Groups Several terms are frequently employed in the various relationships of the blood groups They may be defined as follows

isoimmunization refers to the formation of antibodies when erythrocytes containing specific blood group factors are injected into individuals of the same species who lack this factor

heteroimmunization refers to immunization by injection of red cells into members of another species lacking this factor

blood group typing refers to the determination of blood groups with the use of specific antisera *in transfusions* the importance of cross matching is to prevent the reaction of the donor s red cells to the serum of the recipient

cross matching test for compatibility between the patient s serum and the donor s red cells this is necessary even when the patient and donor belong to the same blood group because of the presence of subgroups and rare types

alleles or allelomorphs alternative forms of genes similar in type but not identical which

occupy the same position on a pair of corresponding chromosomes each chromosome contains a series of places or loci occupied by different genes

homozygous refers to individuals having identical allelic genes on the two corresponding loci of a pair of chromosomes

heterozygous having two different allelic genes on the two corresponding loci of a pair of chromosomes

titer a measure of the relative amount of antibody determined by measuring the highest dilution of serum which can visibly agglutinate cells (in this context erythrocytes)

heterospecific pregnancy a pregnancy in which the fetal red cells possess an A or B factor not present in the mother's cells Also the mother's blood contains an anti A or an anti B agglutinin which is incompatible with the A and B agglutinogen in the red blood corpuscles of the fetus

phenotype the characteristics of an individual represented by direct observation and not revealing all the separate genes responsible for these characteristics

genotype refers to the genetic constitution of an individual for a particular characteristic (in this instance blood groups)

propositus (proband) the original member of the family whose blood group (or other presenting physical or mental characteristic) prompted a genetic or hereditary study

ABO Blood Group System It has been established that the blood of any person falls into one or another of the four well defined major groups according to the antigens in their red cells The presence or absence of each of these blood group factors is determined by their agglutinating reaction with high titered anti A or anti B serum The validity of these tests may be confirmed by adding inactivated serum from the subject to a suspension of known A and B cells Anti A and anti B are almost invariably present in the blood when the corresponding antigen is absent

Distribution of ABO Groups In the United States the incidence of each group is as follows group O 45 per cent A 41 per cent B 10 per cent and AB 4 per cent

Inheritance of ABO Blood Groups Inheritance of ABO groups depends upon three genes A B and O Since an individual inherits the A B or O factor from each parent the combination of genes in the body cells determines the genetic components in the body cells AA AO BB BO AB and OO The A gene occurs in two forms A_1 and A the former being dominant and more frequent Thus about 80 per cent of the persons belonging to group A are included in subgroup A_1 and about 20 per cent in subgroup A With anti A and anti B typing serums the four phenotypes A B AB and O may be detected as shown in Table 1

Table 1 Reactions With Anti A and Anti B Serum

Blood Group	Anti A Serum	Anti B Serum	Serum Isoantibodies (Isoagglutins)
O	−	−	Anti A Anti B
A	+	−	Anti B
B	−	+	Anti A
AB	+	+	

\+ = Agglutination
− = No agglutination

However the blood of persons with genotype AA and that of those with genotype AO cannot be differentiated from each other nor can the blood of persons with genotype BB be differentiated from that of those with genotype BO

Agglutinogens A and B are dominants and O is recessive Several genetic and medicolegal principles stem from the ABO groups For A or B to be inherited at least one parent must transmit either an A or a B gene to the offspring It is apparent that a child cannot belong to group O if either parent belongs to AB for at least one dominant gene A or B must be received from the parent Nor can a child be of any other group but O (genotype OO) if both parents are of that group Also a parent belonging to group O cannot have a child who belongs to group AB

H Substance in Red Cells It is not unusual for O cells to be designated as O (H) H refers to a basic substance from which the A and B substances are made under the influence of the A and B genes H is present in largest quantity in group O red cells and least in A and B red cells [16] A large number of reagents of human animal and even plant origin (such as extracts of Ulex seeds) have been discovered that react with group O cells but not with cells belonging to group A and B It is not possible however to distinguish homozygous AA and BB cells from heterozygous AO and BO cells by titration against the corresponding A or B isoagglutinins

Development of Blood Groups A and B isoagglutinogens can be demonstrated in the fetus in the second and third months of life [13] Anti A and anti B isoagglutinins found in the cord blood diminish in titer or completely disappear in the first weeks or months of life That these isoagglutinins have been passively transferred from the mother is evident from the similarity existing between the blood of the newborn infant and that of the mother with respect to isoagglutinins [19] The isoagglutinins in the newborn infant s serum are therefore not its own but are derived from the mother by filtration through the placenta In many pregnancies however the placenta exerts a definite limiting effect upon the extent to which the maternal agglutinins can enter the baby s circulation [0] Between 3 and 6 months of age the child begins to make its own isoagglutinins The strength of the isoagglutinins rapidly rises and is at its maximum at 5 to 10 years of age after this there is a gradual fall [17] The anti A titers are higher than anti B

Secretors and Nonsecretors The blood factors A B and O are not restricted to the red cells since in approximately 80 per cent of human beings they are also present in water soluble form in the body fluids and in such secretions as saliva gastric juice nasal secretion semen and amniotic fluid [3] Persons who have blood group substances present in their secretions are known as "secretors" and those who do not are known as "nonsecretors" The blood group substances (agglutinogens) A and B are stable polysaccharide-amino acid complexes containing galactose and an amino sugar N acetyl glucosamine The classification of a person as a secretor or a nonsecretor is most readily accomplished by testing the saliva where blood group substances are present in high concentration It has been shown that practically all nonsecretors of A B and H substances are also positive for the Lewis (Le) blood factor (See discussion of the Lewis group later in this chapter)

Universal Donor and Recipient Since the red cells of group O are not agglutinated by any serum the persons belonging to this group are termed universal donors However some group O blood may contain such a high titer of anti A and anti B agglutinins that despite dilution in the recipient s blood the titer may remain sufficiently elevated to cause serious red cell destruction Generally such blood is considered safe if the titer is not over 1 to 100 Blood group specific substances A and B (Witebsky substance) are used to neutralize the anti A and anti B isoagglutinins of group O blood before it is given to persons of other blood groups One unit (vial) is sufficient to reduce the anti A and anti B in 500 ml of blood to at least one fourth of its original titer[4] When group AB blood is not available for AB recipients experience has shown that lower titer group O blood is satisfactory It has been estimated that the serum of 3 per cent of persons belonging to group AB contains anti O

Rh Hr Blood Group System The discovery of the Rh factor provided an explanation for the cause of transfusion reactions other than those due to the four major blood groups of the ABO system described by Landsteiner In 1940 Landsteiner and Wiener[9] discovered the Rh factor They found that when the blood of the rhesus monkey was injected into a rabbit the serum contained an antibody which not only agglutinated rhesus but human red cells as well This factor corresponded in activity to one postulated previously by Levine and Stetson[1] to explain the cause of a severe reaction to the initial blood transfusion in a woman recently after delivery of her baby

Further investigation revealed that the Rh factor was present in 85 per cent of the white population People having this agglutinogen were designated Rh positive whereas those in whom it was absent were designated Rh negative In the Negro population the percentage of Rh positive is 93 per cent and in the Chinese it is 99 per cent There are no naturally occurring antibodies to the Rh factor in contrast to their presence in the A and B groups The Rh agglutinins may be produced however when an Rh negative person receives Rh positive blood

A basic group of three sets of alternative antigens has been described within the Rh system The capacity of a red cell to react with a given antiserum is based on the presence of a specific antigen in the cell When one of the Rh antigens cannot be demonstrated as indicated by a negative reaction with its antiserum another contrasting or reciprocal antigen (Hr) is present in its place Thus every person inherits one set of three Rh genes or corresponding Hr genes from each parent

C D E Notations for Blood Groups The symbols C c D d E and e are used for the blood antigens detected by appropriate antiserum the small letters corresponding to the Hr factors Thus there are two sets of terminologies in relation to the Rh Hr system An allelomorphic relationship exists therefore between rh and hr′ Rh and Hr rh and hr′ (C and c D and d and E and e respectively)

Genetics of the Rh Hr Blood Types According to Wiener [4] single Rh Hr genes with a series of alleles at one locus on the chromosome determine the presence of these factors in the blood According to the Fisher Race concept[14] on the other

Table 2 Six Basic Rh Hr Factors as Determined by Their Corresponding Antisera

Wiener System	Fisher Race System
rh	C
Rh	D
rh	E
hr	c
Hr	d
hr'	e

hand each Rh blood factor is controlled by an individual gene in a series of pairs of alleles each at one of three adjacent closely linked loci on one of the twenty four human chromosomes [2]

The six basic Rh Hr factors as determined by their corresponding antisera may be listed as shown in Table 2

Since the Rh Hr genes are alleles occupying the same locus or position of corresponding chromosomes a person must have an Rh antigen or one Hr antigen or both Thus Rh testing with specific serums may reveal that the person has rh rh (homozygous) or rh hr (heterozygous) A person who is Rh negative must therefore be cde/cde Rh positive persons on the other hand may possess a wide variety of genotypes the more common being CDe/cde CDe/CDe CDe/cDE and cDE/cde

Using the Fisher Race nomenclature for the gene D or its allele d there are three possible genotypes DD Dd and dd If anti d serum were available all three could be recognized Since only anti D is available no distinction can be made between DD and Dd and both are similarly agglutinated

There are three principal Rh factors Rh present in approximately 85 per cent of Caucasian people rh present in 70 per cent and rh present in 30 per cent The symbol Rh has a capital Rh to indicate its special serologic and genetic position whereas rh and rh have a small r to indicate their lesser importance

*Table 3 The Eight Standard Rh Allelic Genes**

Gene	Corresponding Agglutinogen	Blood Factors Present	
r	rh	hr' and hr	Rh negative aggutinogens
r	rh	rh and hr	
r'	rh	rh" and hr'	
r	rh	rh and rh	
R	Rh	Rh hr' and hr"	Rh positive agglutinogens
R	Rh	Rh rh and hr	
R	Rh	Rh rh and hr'	
R	Rh	Rh rh and rh	

* From Wiener A S and Wexler I B Heredity of the Blood Groups New York 1958 Grune & Stratton Inc p 75

clinically For some reason the Rh_1 factor is a more potent producer of antibodies than are rh rh hr hr′ or Hr_0 The rh negative person is represented by rh since the cells do not react with anti Rh_1 anti rh and anti rh serums Except for anti d (Hr) testing serums are available for each of the Rh Hr factors Despite this deficiency it is possible to hazard the presence of homozygosity or heterozygosity in a person on the basis of the statistical frequency of the reaction of the subject s cells to the available antiserums

For routine clinical use it is sufficient to subdivide human beings into Rh positive and Rh negative according to their reactions with the single anti Rh (anti D) serum By this means it is found that of all of those who become immunized to Rh whether by transfusion or pregnancy approximately 99 per cent are Rh negative[16]

The Rh Hr system receives its widest clinical application in erythroblastosis fetalis Rh (D) is the most antigenic factor and is involved in approximately 93 per cent of cases of this disease

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In addition to the ABO and Rh Hr blood group systems there are other red cell antigens of clinical importance such as Kell and Duffy Still others (MN Ss P Lutheran and Lewis) are rarely if ever involved in human disease but are employed in precise identification of the red cell

MN, Ss and P Blood Group Systems The MN Ss and P blood group systems are rarely involved in intra group incompatibilities In addition to their use in the investigation of the cause of obscure transfusion reactions they provide useful tools in human genetics and with the major blood groups help to resolve medi colegal problems of identity and parentage

The M N and P agglutinogens were discovered by Landsteiner and Levine[8] who used the serums of rabbits immunized with human red cells After absorption of species agglutinins the serums agglutinated some samples of human blood but not others Naturally occurring antibodies against M and N are infrequent in the human being so that their detection requires the use of rabbit immune serums Like A and B antigens M and N are well established in the newborn infant Three genotypes occur MM MN and NN Type MN is found in 50 per cent of human beings M in 30 per cent and N in about 20 per cent A few human beings with acquired anti M antibodies have been encountered but these rarely lead to transfusion reactions or erythroblastosis N is a much weaker antigen and has not been responsible for any difficulties

Blood cells possessing the P factor are designated P+ and those lacking it P− Naturally occurring anti P agglutinins can be found in a few unselected P negative persons Anti P isoimmunization is not known to cause erythroblastosis and rarely causes hemolytic transfusion reactions

The antigenic factor S[1] and related antigen s are also present in human blood Cases of erythroblastosis fetalis and hemolytic transfusion reactions have resulted from antigenic stimulation by anti S[3] S bears a close genetic relationship to M and N[17] and is probably closely linked with them on a single chromosome A number of examples of anti S antibodies occurring spontaneously have been re ported but more commonly they result from immunization

Rh Variants Rh variants include the D factor C factor Kell groups (Kk) Duffy group (Fy^a and Fy^b) Lewis group (Le^a and Le^b) Lutheran group (Lu and Lu^b) Kidd group (Jk and Jk^b) factor U and low incidence antigens

D Factor D is a modified D (Rh_0) antigen which reacts weakly or not at all when tested with some anti D (Rh_0) serums It is for this reason that such blood is occasionally classified as Rh negative Its positive Rh character can be recognized by its reaction to high titered anti D serum and the indirect Coombs test In the person with the D^u factor exposure of the blood to immune (incomplete) anti D serum will result in agglutination with antiglobulin (Coombs) serum To avoid overlooking the D^u variant all blood designated as Rh negative should be subjected to additional screening by the indirect Coombs test

C^w Factor The Rh antigen C^w (rh^w) is a third allelomorph at the C locus *Anti C^w antibodies result from* transfusion with C^w blood and from isoimmunization in pregnancy [15] causing erythroblastosis fetalis

Kell Groups (Kk) The Kell Duffy Lewis Lutheran and Kidd factors derive their names from the patients involved in cases of erythroblastosis fetalis and transfusion reactions and from prospective donors

The Kell (K) and Cellano (k) factors are alleles with equal dominance The Kell antibody has been responsible for many cases of transfusion reactions and erythroblastosis In these respects it is secondary in importance only to the antibodies against D (Rh) A and B On the other hand the Cellano (k) factor which is the reciprocal of Kell is of minor importance No naturally occurring Kell antibodies have been described

Duffy Group (Fy and Fy') For the Duffy Lewis Kidd and Lutheran systems a combination of two letters is used to indicate the blood group system and the particular gene or antigen *is further designated by adding a b etc as* a superscript [16]

The Duffy system (Fy) has been shown to be responsible for hemolytic transfusion reactions but not as yet for erythroblastosis fetalis Fy and Fy^b constitute two of the better known alleles of this factor Anti Fy antibody is by far the more common of the two

Lewis Group (Le and Le') Only a few cases of hemolytic transfusion reactions have been caused by anti Le [1] It is of interest that practically all persons whose red cells are Lewis positive are also salivary nonsecretors of AB and H substances and vice versa Thus anti Le^a serum can be used to detect the non secretor of group A B and O [6] Rosenfield and Ohno (cited by Mollison[16]) have also pointed out that the Lewis phenotype appears as an agglutinable characteristic of the red cells only after a few weeks of life possibly because the Le and Le^b factors are absent or extremely weak at birth

Lutheran Group (Lu and Lu^b) The Lutheran blood group system occurring in 6 per cent of the English population has no clinical importance The Lutheran antibody has been detected in Lutheran negative subjects who have received multiple transfusions This group plays no part in hemolytic transfusion reactions or erythroblastosis

Kidd Group (Jk^a and Jk^b) Anti Jk was initially described in the blood of a patient who had given birth to an infant with erythroblastosis [1] This patient had

never had a transfusion There is as yet no conclusive evidence that this antibody is responsible for hemolytic transfusion reactions The allelomorph Jk^b discovered later occurs even more rarely The antibodies of the Kidd group are usually detected by the indirect Coombs test but preliminary application of trypsin to the red cells may be necessary

Factor U The U factor was originally described in a patient with a fatal transfusion reaction [3] This factor is almost universally present in human erythrocytes The few persons who are negative to this factor possess blood of type N or MN [4] Sensitization to the U factor is extremely rare and it is of interest that all clinical cases have been reported in Negroes Of Negroes tested 1 2 per cent are U negative hence the possibility of sensitization by transfusion [18]

Low Incidence Antigens ("Private Blood Group Antigens) Infrequent cases of intragroup immunization to a variety of rare antigens constitute a miscellaneous group known as family antigens" or "private" blood group antigens [10] Here the positive or negative reactions are linked solely to members of a particular family They are usually discovered in the investigation of cases of erythroblastosis fetalis in which the etiology is not accounted for by established blood groups Of special interest is the Jay antigen (Tj) In five married women of child bearing age each of eighteen pregnancies ended in a miscarriage at two to five months gestation [11] because of incompatibility within this system

REFERENCES

1 Allen F H Diamond L K and Nedzieli B A New Blood Antigen Nature 167 482 1951
2 Boyd W C The Inheritance of Blood Groups Am J Clin Path 26 654 1956
3 Brandes W W Cahan A C and Jack J A Incompatible Transfusion Caused by anti S J A M A 156 836 1954
4 Bray W E Clinical Laboratory Methods ed 5 St Louis 1957 The C V Mosby Co
5 Carpenter P L Immunology and Serology Philadelphia 1956 W B Saunders Co
6 Grubb R Correlation Between Lewis Blood Group and Secretor Character in Man Nature 162 933 1948
7 Kabat E A Blood Group Substances New York 1956 Academic Press Inc
8 Landsteiner K and Levine P A New Agglutinable Factor Differentiating Individual Human Bloods Further Observations on Individual Differences of Human Blood Proc Soc Exper Biol & Med 24 600 1927
9 Landsteiner K and Wiener A S An Agglutinable Factor in Human Blood Recognized by Immune Sera for Rhesus Blood Proc Soc Exper Biol & Med 43 223 1940
10 Levine P A Brief Review of the Newer Blood Factors Tr New York Acad Sc 13 205 1951
11 Levine P and Koch E A The Rare Human Isoagglutinin Anti Tj and Habitual Abortion Science 120 239 1954
12 Levine P and Stetson R An Unusual Case of Intra group Agglutination J A M A 113 126 1939
13 Levine P Burnham L Katzin F M and Vogel P The Role of Isoimmunization in the Pathogenesis of Erythroblastosis Fetalis Am J Obst & Gynec 42 925 1941
14 Levine P Vogel P and Rosenfield R E Hemolytic Disease of the Newborn In Levine S Z (editor) Advances in Pediatrics Chicago 1953 Year Book Publishers Inc p 97
15 Matson G A Coe J and Swanson S Hemolytic Transfusion Reaction Due to Anti Le Blood 10 1236 1955

16 Mollison P L Blood Transfusion in Clinical Medicine ed 2 Springfield Ill 1956 Charles C Thomas Publisher
17 Race R R and Sanger R Blood Groups in Man ed 2 Springfield Ill 1954 Charles C Thomas Publisher
18 Sacks M O Schultz C Dagovitz L and Vanecko M Hemolytic Disease of the Newborn Due to the Rare Blood Factor rh^w (C^w) Pediatrics 21 443 1958
18a Sampson C C Thomas C and Griffin S Isosensitization to the U Factor J A M A 171 1203 1959
19 Smith, C H Isoagglutinins in the Newborn With Special Reference to Their Placental Transmission Am J Dis Child 36 54 1928
20 Tovey G H A Study of the Protective Factors in Heterospecific Blood Group Pregnancy and Their Role in the Prevention of Haemolytic Disease of the Newborn J Path & Bact 57 295 1945
21 Walsh R J and Montgomery C M A New Human Isoagglutinin Subdividing the MN Blood Groups Nature 160 504 1947
22 Watkins W M and Morgan W T J Some Observations on the O and H Characters of Human Blood and Secretions Vox Sang 5 1 1955
23 Wiener A S Blood Groups and Transfusion ed 3 Springfield Ill 1943 Charles C Thomas Publisher
23a Wiener A S Unger L J and Gordon E B Fatal Hemolytic Transfusion Reaction Caused by Sensitization to New Factor U J A M A 153 1444 1953
24 Wiener A S and Wexler I B Heredity of the Blood Groups New York 1958 Grune & Stratton Inc

CHAPTER 7

*Transfusions in Pediatric Practice**

Significant Factors in Transfusion Therapy The approach to transfusion in the pediatric patient necessitates certain background data in order to evaluate the need of transfusion in relation to the growth period potential risks and technicalities of administration Such data summarized as follows also provide a basis for the indications and certain of the limitations of transfusion therapy

Blood volume—80 ml per kilogram
Plasma volume—45 ml per kilogram
Erythrocyte volume—35 ml per kilogram
Hemoglobin concentration—12 to 13 gm per 100 ml
Hematocrit—36 per cent
1 gm hemoglobin = 3 4 mg iron
100 ml transfused whole blood (average)—50 mg iron
500 ml transfused whole blood (average)—250 mg iron

Dosage for transfusion

Whole blood
- 20 ml per kilogram when body weight is less than 25 kg
- Units of 500 ml when body weight is more than 25 kg

Packed or sedimented erythrocytes
- 15 ml per kilogram when body weight is less than 20 kg
- Units of 300 ml when body weight is more than 20 kg

Plasma—10 to 15 ml per kilogram

Indications for Transfusion The indications for transfusion are listed as follows

To restore deficit of hemoglobin and erythrocytes
- Acute and chronic blood loss
- Hemolytic anemias—congenital and acquired
- Defective blood formation
- Pre and postoperative

To restore blood volume
- Shock burns trauma

To supply specific coagulation factors including platelets

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* Quoted in part from Smith C H Transfusions in Pediatric Practice Indications and Limitations Pediatrics 17 596 1956

Miscellaneous
- Infections
- Hypoproteinemia
- Exchange in erythroblastosis
- Poisoning

In pediatric practice the indications usually apply to treatment of patients with blood disorders infection and nutritional and deficiency states In contrast to the situation in the adult the management of transfusion needs in the child requires the additional appraisal of abnormality in relation to a specific stage of growth and development For instance prescribing a hemoglobin level to be achieved by transfusion may not be a simple task in the infant or young child because the optimal value is not fixed but is subject to individual interpretation

Blood Volume—Plasma, Total Circulating Hemoglobin, and Erythrocyte Mass A knowledge of the shifts in blood volume has become almost mandatory in the interpretation of many clinical syndromes and in providing clues for their treatment The need for recognizing fluctuations of blood volume (plasma total circulating hemoglobin and erythrocyte mass) was realized early in patients in surgical shock so that adequate physiologic restitution could be planned Acute hemorrhage associated with blood disorders or accompanying shock burns or organic lesions calls for transfusion of calculated amounts of whole blood red cell suspensions or plasma when feasible Blood volume estimations are now used in the treatment of patients with erythroblastosis in whom an exchange of at least twice the estimated volume represents current practice

Comparisons in terms of total hemoglobin and erythrocyte mass permit evaluation of therapeutic procedures or the need for such procedures by eliminating the influence of variations in plasma volume The determination of total circulating hemoglobin and erythrocyte content therefore has come into greater use because it frequently obviates erroneous conclusions drawn from a peripheral blood sample From the blood volume (80 ml per kilogram of body weight) and hemoglobin measurements (expressed as grams per 100 ml) the total circulating hemoglobin mass can be readily determined In patients in whom transfusions are being considered periodic estimations of the absolute hemoglobin mass frequently prove more valuable in detecting the early regeneration of hemoglobin than in determining the peripheral hemoglobin concentration This calculation which takes into account the factors of growth and hemodilution has proved helpful in recognizing the initial states of hemoglobin regeneration in the premature infant[18] and in evaluating the hemoglobin level prior to transfusion in the patient with refractory chronic hemolytic anemia such as Mediterranean anemia [19] In erythroblastosis for instance transfusions can be postponed in patients with hemoglobin concentrations of 8 gm per 100 ml at the fourth to sixth week of life in the so called convalescent anemic phase[16] when it is found that the total circulating hemoglobin mass either has stabilized or has begun to rise

With certain exceptions a blood volume of 80 ml per kilogram of body weight

applies throughout life In the newborn infant the value of 85 ml per kilogram has been found more accurate in calculating the blood volume prior to administration of exchange transfusion In our experience the blood volume has been found to exceed the normal with values as high as 104 ml per kilogram in a small number of patients with sickle cell anemia who were not in crisis An inordinate increase in plasma volume was responsible for such values This hemodilution probably accounted for the freedom from disability of these patients since their hemoglobin values were maintained without transfusions at levels of 6 to 7 gm per 100 ml In our experience crisis is associated with a sharp decrease in plasma volume but the amount of hemoglobin undergoes little or no change In an attempt to restore the expanded precritical plasma volume whole blood and plasma have been given with moderate success Increased fluid intake either orally or parenterally is a valuable adjunct

Hemoglobin and Hematocrit Levels in Acute and Chronic Anemias In the management of severe anemia the objective in the restoration of a normal level of hemoglobin is different in acute blood loss than in chronic anemia In the patient with nonrecurring hemorrhage or in the infant with severe iron deficiency who is too ill to await the effects of specific therapy a sufficient amount of whole blood or preferably packed erythrocytes may be given to achieve hemoglobin levels of 12 to 13 gm per 100 ml and a hematocrit of 36 per cent

The transfusion of packed erythrocytes to the patient with chronic anemia is to be contrasted with the need for rapid transfusion of whole blood to the patient with acute hemorrhage Provided the cause of acute hemorrhage is corrected the bone marrow will be adequate to maintain normal blood levels subsequently In patients with chronic hemorrhagic disorders completely normal levels of hemoglobin *need* not be attained with each transfusion if large amounts of blood are required to accomplish this end A normally functioning bone marrow will eventually raise blood levels to desired values

The hemoglobin value at which a transfusion is indicated in patients with anemias of the aplastic hypoplastic group or with chronic hemolytic anemias such as severe Mediterranean anemia and sickle cell anemia cannot be arbitrarily fixed rather it varies with associated signs and symptoms of the individual patient As a guide to management patients with the aplastic hypoplastic and chronic hemolytic anemias usually do not require transfusions until hemoglobin levels decrease to 7 to 7 5 gm per 100 ml at which point clinical symptoms usually appear Whereas blood levels in children with the aplastic hypoplastic anemias and *in those with severe Mediterranean anemia tend to decline progres*sively unless treatment is given the patient with sickle cell anemia reacts differently In the latter the need for repeated transfusions is much less urgent because hemoglobin levels usually stabilize in a range between 6 and 7 gm per 100 ml without discomfort or interference with activities and regardless of treatment Although these criteria find general application the amounts of blood given and the intervals between transfusions require specialized study for each patient

Choice and Dosage of Whole Blood Packed Erythrocytes and Plasma Although the choice and dosage of whole blood packed erythrocytes and plasma

are frequently clear cut they often depend upon the appraisal of factors other than the hemoglobin level and the erythrocyte count A large body of experience has demonstrated the superiority of whole blood especially when blood volume and anemia *require simultaneous correction In patients with disorders of the* clotting mechanism fresh whole blood is of value in supplying both the appro priate plasma factors and the erythrocytes lost by hemorrhage Whole blood plasma or a combination play a part in the treatment of dehydration diarrhea and protein deficiency The immunologic support represented by the transfer of circulating antibodies and gamma globulin in combating infection has now been replaced by the antibiotics and when needed by the gamma globulin fraction directly

The administration of packed erythrocytes by permitting a larger dose in a single transfusion represents an ideal treatment in infants in patients with cardiac failure and in patients with chronic anemias refractory to other forms of therapy To obtain concentrated suspensions of red cells plasma is removed from sedimented cells or after centrifugation However in bleeding episodes associated with secondary thrombocytopenia due to bone marrow depression such as occur in patients with aplastic anemia and leukemia especially during chemotherapy fresh whole blood is preferable especially for its platelet con tent and possibly other constituents of plasma Although the transfer of platelets may not be so effective as when concentrated platelet suspension is used[1] fresh whole blood is more easily obtainable and frequently controls hemorrhage satisfactorily

Fresh platelet rich plasma collected in plastic bags and administered directly *after bleeding the donor has proved to be one of the most effective means of* controlling hemorrhage in patients with primary or secondary thrombocytopenia Blood platelets drawn in this manner have a survival span of 4 to 6 days For the actively bleeding patient with leukemia the continuous transfusion of platelet rich plasma and fresh whole blood checked by repeated hemoglobin determina tions has been found to be one of the best methods of controlling severe epistaxis and bleeding from the gastrointestinal tract When available platelet rich blood from a polycythemic donor given by direct transfusion through silicone coated syringes is ideal treatment for restoring both intact platelets and red blood cells [1] Adrenocorticotrophic hormone (ACTH) and steroids (prednisone predni solone and hydrocortisone) given intravenously also serve as valuable hemo static agents This form of therapy is especially indicated in patients with idio pathic thrombocytopenic purpura in the active state in patients being prepared for splenectomy and also in controlling hemorrhage in patients with secondary thrombopenic states

Plasma transfusions restore blood volume when whole blood is not available correct a variety of clotting deficiencies (see Chapter 26) and supply protein in patients with hypoproteinemia In treating patients with classical hemophilia individual units of fresh frozen group specific plasma are superior to pooled plasma in diminishing the risk of hepatitis [3]

In regulating the amounts of blood for transfusion it should be remembered that citrated (ACD) blood usually has a hematocrit of 32 to 36 per cent as

compared with 40 to 50 per cent for whole blood and 60 to 70 per cent for packed erythrocytes depending upon the amount of supernatant plasma removed in the latter [14]

The dosages listed on p 75 serve as guides for the amount of blood and plasma to be given in a single transfusion In the case of whole blood and packed erythrocytes the values are calculated to elevate the hemoglobin concentration and erythrocyte count in varying degree depending upon the severity of the anemia but within safe limits of blood volume

With increasing weight and expanding blood volume these figures lose their significance and other criteria are substituted depending upon the objectives of therapy such as hemoglobin elevation cessation of hemorrhage and clinical improvement It should be obvious that the older child with chronic anemia who at irregular periods receives a single transfusion of 500 ml of whole blood and lesser amounts of packed erythrocytes cannot be expected to show a significant rise in hemoglobin The amount of blood is necessarily diluted in a higher blood volume than it is in the smaller child to whom similar amounts may be given Hence in determining the rate of survival of donor blood several transfusions are necessary within a short period of time to raise blood values to normal levels from which the rate of decline may be estimated

Transfusions in Premature Infants In most clinics a conservative policy is followed with regard to transfusions in the healthy premature infant At the age of 4 to 7 weeks when the infant s fetal and adult hemoglobin values have decreased to low levels spontaneous recovery usually begins with the predominant synthesis of the adult type [15] Administration of transfusions at a time when normal regeneration of hemoglobin is at hand seems unnecessary Besides the known complications to give transfusions at this point is to run the risk of depressing inherent bone marrow function [3,15]

The present trend as disclosed in a survey [11] is to give transfusions to only those premature infants whose hemoglobin values have decreased to between 6 and 9 gm per 100 ml Our own practice has been to give transfusions to only those whose hemoglobin concentrations persist at levels of approximately 7 to 8 gm per 100 ml in the absence of significant reticulocytosis Transfusion is employed when there are evidences of infection anorexia listlessness and failure to gain weight We have found that premature infants rarely need transfusions on the basis of anemia alone When transfusions are required either packed erythrocytes or whole blood is given in amounts not to exceed 10 ml per kilogram of body weight

Limitations and Hazards of Transfusions Increased experience and investigation of untoward reactions have provided a greater measure of safety in the administration of transfusions Of the limitations and hazards included in the following list the most common are the hemolytic reactions serum hepatitis allergic and febrile reactions and circulatory overload

Development of intragroup isoimmunization
Hemolytic reactions
    Incompatible blood
    Old stored blood

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of transfusion to produce an anticipated rise in hemoglobin and erythrocytes in the absence of hemolysis or hemorrhage

Two recent sets of observations[19, 7] document the thesis that transfusions administered at frequent intervals retard hemoglobin and erythrocyte formation These data are based on an intensive study of a small group of children with severe Mediterranean anemia in whom transfusions could be interrupted following splenectomy By the methods designed to permit quantitative differentiation between donor and recipient blood and by serial examinations of the total erythrocyte mass and circulating hemoglobin it was possible to demonstrate that a retarding effect occurred which was most marked from the first to the third week following transfusion Not until donor blood had been entirely eliminated from the circulation were pretransfusion blood levels restored in the patient The depressant effect of transfusion was further substantiated by another means In patients with severe Mediterranean anemia the presence of large amounts of fetal hemoglobin provides a biologic tag of endogenous hemoglobin synthesis Transfusions resulted in a depression of this component which increased sharply as donor cells left the circulation The diminution in circulating fetal hemoglobin persisted in one patient for the first seven weeks after transfusion and in two others the decrease amounted to 50 per cent Paralleling these observations is a recent report[7] that in patients with sickle cell anemia who received multiple transfusions a period of maximum depression of erythropoiesis occurred from the twelfth to the twenty fifth day with the percentage of sickle cells decreasing from 100 to 5 per cent It is therefore advisable to withhold treatment occasionally in patients with chronic anemia to determine innate bone marrow function In critical periods of growth such as in the anemia of prematurity it may be undesirable to interfere with bone marrow function by transfusion In patients with erythroblastosis it has been suggested that a similar retardation can result from a persistent effort to maintain normal blood levels These studies suggest the need for a less empirical and more individualized orientation not only in patients with refractory anemias but also in those with other disease conditions requiring frequent transfusions The spacing and size of transfusions and blood levels to be attained require constant re examination and appraisal

Transfusions and Hemosiderosis Recent studies in iron metabolism have extended into an investigation of "iron overload or hemosiderosis resulting from repeated transfusions The total amount of iron in a normal adult approximates 4 to 5 gm of which 15 to 20 per cent represents the iron reserve stored principally in the liver as ferritin and hemosiderin In children storage iron normally represents 8.5 to 10 5 mg per kilogram of body weight In states of iron deficiency in infancy due to inadequate diet and periods of rapid growth storage depots are taxed and may be depleted Iron otherwise accumulates and except for the normal excretion of approximately 1 mg daily cannot leave the body except by bleeding From the list on p 75 it is apparent that excessive amounts of iron from destroyed erythrocytes become available for storage in patients receiving multiple transfusions (250 mg of iron in 500 ml of blood) Under such conditions hemosiderin deposits in normal storage depots increase greatly and in addition accessory sites assume the function of iron storage Hemo

- Transmission of disease
  - Homologous serum jaundice (serum hepatitis)
  - Syphilis
  - Malaria
- Circulatory overload
- Excessive iron deposition
  - Hemosiderosis
  - Exogenous *hemochromatosis*
- Suppression of hematopoiesis
- Miscellaneous
  - Febrile reactions
  - Allergic reactions
  - Reactions from infected blood
  - Potassium toxicity
  - Cold agglutinins
  - Air embolism
  - Citric acid intoxication

Many advances have been made in the direction of reducing the icterogenic properties of plasma such as reduction of the size of the pool ultraviolet irradiation and the storage of liquid pooled plasma for prolonged periods at room temperature[*] The incidence of isosensitization from blood transfusion has been markedly reduced by preliminary screening and cross matching refinements of techniques and identification of increasing numbers of rare blood factors

Circulatory Overload The administration of excessively large quantities of blood or even smaller amounts if given rapidly may precipitate cardiac failure from circulatory overload In the course of a transfusion or shortly thereafter precordial pain dyspnea cyanosis and a dry cough are indicative of a rising venous pressure and pulmonary edema In patients with severe anemia and evidences of congestive heart failure preliminary digitalization occasionally may be required Packed or sedimented red cell suspensions are useful whenever it is essential to provide blood with the least possible disturbance to the patient's blood volume Another expedient to prevent overloading the circulatory system is to give one half the circulated amount of blood in successive days

In erythroblastosis death may result from cardiac failure caused by administering more blood than has been removed Preliminary withdrawal of 30 to 50 ml of blood from the severely anemic infant may be necessary[14] or a deficit of even larger amounts (40 to 80 ml) should be established within a short time after beginning the exchange when venous pressures are excessively high[1]

Transfusions and Suppression of Blood Production One of the limitations of transfusion which deserves comment and which has as yet received scant attention is its potential depressant effects upon endogenous erythropoiesis and hemoglobin synthesis That transfusions possess this secondary inhibitory effect was recognized in the treatment of pernicious anemia before the advent of specific therapy and has been confirmed more recently in several studies[4,6,10,1] Although retardation of hematopoiesis in minor degree may accompany a single transfusion it is overshadowed by the major corrective effects In the case of multiple transfusions however this limiting influence may explain the failure

Transfusion Therapy in Hemorrhagic Disorders The intensive investigations of the hemorrhagic disorders have led to a clearer definition of the role of established and newly discovered factors in maintaining hemostasis With the application of specialized procedures defects heretofore unknown have been described and appropriate treatment advised These will be discussed under hemorrhagic diseases in Chapter 26 In general the patients with hemorrhagic disorders will benefit from fresh whole blood or plasma until the specific defect is determined

Notes on Technique and Preservation of Blood When siliconized equipment is available its use is desirable for collection and administration of blood A 20 gauge by 1 inch needle with a short sharp bevel is used in transfusion of patients in any age group

In young children who require multiple transfusions such as those with leukemia or aplastic anemia suitable superficial veins often eventually become inaccessible or thrombosed To avoid a cut down which may be accompanied by persistent oozing whole blood or plasma can be transfused into the proximal end of the tibia several inches below the tubercles An appropriate bone marrow needle usually 18 gauge by 1 inch is inserted into the marrow as for aspiration and the distal plastic tip of the administration set is placed directly into the marrow needle The flow is regulated as for intravenous administration with the bottle suspended at a level permitting flow by gravity Because of the danger of infection it should be emphasized that this method of administering blood is used only as a last resort

Acid citrate dextrose solution (ACD) is now generally used as an anticoagulant The inclusion of dextrose in the preservation of blood slows the fall of diphosphoglycerie acid and adenosine triphosphate (ATP) The latter are involved in the energy producing mechanism of the red cell and permit its longer survival by maintaining red cell integrity

During blood storage the leukocytes quickly disintegrate and lose their phagocytic power The platelets disappear in about four days antihemophilic globulin is progressively decreased[15] (in some instances as much as 10 per cent in the first week and approximately 50 per cent of the initial antihemophilic globulin after three weeks storage) and the prothrombin time is prolonged due to the loss of factor V The use of plastic (Fenwall) bags retards the destruction of platelets Platelet rich plasma obtained by this means has become increasingly popular in the treatment of serious bleeding in patients with thrombocytopenic purpura and leukemia

In blood stored under refrigeration the potassium content of red cells decreases and that of the plasma increases both factors being intensified during prolonged storage Marked electrocardiographic changes do not occur until the potassium level reaches approximately 8 mEq per liter[11] Hyperkalemia induced in patients during exchange transfusions is transient but the potential risk of toxic effects should be kept in mind The latter can be minimized by the use of relatively fresh blood the removal of part of the plasma and the judicious use of calcium during replacement transfusions with citrated whole blood[13]

siderosis refers to increased iron stores without tissue damage; hemochromatosis to the development of tissue damage in persons with prolonged iron excess (see Chapter 12). The amount of iron present in organs such as the liver may exceed the amount given by repeated transfusions, indicating excessive absorption of iron from the gastrointestinal tract.[3]

Whether iron from transfused blood actually may produce hemochromatosis is still controversial, but there is evidence that excessive iron storage is potentially injurious.[9] It must be understood that no unequivocal statement can be made as yet as to the extent or manner in which tissue siderosis is harmful. Studies in severe Cooley's anemia[4] have shown, however, that hemosiderosis and fibrosis frequently coexist, although there was no uniformity about the progression.

The development of true hepatic cirrhosis and fibrosis of the pancreas which characterizes hemochromatosis associated with transfusion hemosiderosis may depend upon the intervention of accessory factors such as continued hypoxia in addition to large iron deposits. Whether iron deposits alone are responsible for the hepatic damage in hemochromatosis is still debatable, but that it is a prerequisite for this pathologic process is more likely.

From these considerations it would seem that the iron derived from an occasional transfusion is harmless. A contrary situation may prevail, however, in the patient receiving multiple transfusions over long periods of time. In view of the risk involved, the most prudent course is to restrict the number of transfusions to the minimum compatible with comfort and moderate activity.

Allergic Reactions. It has been estimated that about 1 to 5 per cent of blood transfusions are followed by allergic reactions, principally urticaria and less often angioneurotic edema and asthma. These reactions are especially frequent *in children with refractory anemias requiring multiple transfusions.* In our experien

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Smith, Carl H. *Blood Diseases of Infancy and Childhood*

Erratum—Page 82

In the last line of the text and in the second line of the title to Table 4 *exchange transfusions* should read **multiple transfusions**

Table 4

Allergic Reactions to Exchange Transfusions

Aspirin	
Up to 5 years	1 grain (60 mg.) per year
5 to 10 years	10 grains
Tripelennamine (Pyribenzamine)	
Up to 5 years	25 mg.
5 years and over	50 mg.
Prednisone	
Regardless of age	10 mg. given ½ to 1 hour before transfusion

Other antihistamines may be substituted for Pyribenzamine

CHAPTER 8

Jaundice—Differential Diagnosis in the Neonatal Period

The differential diagnosis of jaundice in the first weeks and months of life embraces a variety of conditions of a hemolytic and hepatic nature some of which are specific and others ill defined These conditions may be interrelated to the extent that they appear as a hemolytic process at the outset but eventually give the clinical picture of liver disease Common to most of these conditions in the first days of life is accelerated red cell destruction resulting in overproduction of bilirubin in amounts exceeding the excretory capacity of the liver for their disposal On the other hand jaundice may date from the newborn period on a purely hepatic basis without significant intensity until the second or third week of life Here malformations of the bile ducts or an inflammatory process accounts for the jaundice uncomplicated by a hemolytic component

Erythroblastosis plays a prominent role in the differential diagnosis of jaundice both in the neonatal period when the disease is active and in the early months of life when it is an important factor in the pathogenesis of the inspissated bile syndrome Since erythroblastosis will be dealt with fully in Chapters 9 and 10 in this discussion it will be referred to only in connection with syndromes from which it needs to be differentiated

The pediatrician is confronted with making a diagnosis in infants with syndromes characterized by jaundice either during the first week of life (early neonatal period) as in those with erythroblastosis or in the remainder of the neonatal period (second to fourth weeks of life) as in those with prolonged obstructive jaundice

Types of Bilirubin Different types of bilirubin have been identified with each process on the basis of their reaction with van den Bergh s diazo reagent The serum from patients with bilirubinemia resulting from red cell destruction produces a predominantly delayed or indirect reaction whereas the serum from those with jaundice due to diseases of the liver and biliary system produces a direct (one minute) reaction Bilirubinuria is absent in patients with hemolytic anemia in whom there is a retention of nonconjugated bilirubin (water insoluble form) in the plasma giving an indirect van den Bergh reaction In patients with

REFERENCES

1 Allen F H Jr and Diamond L K Erythroblastosis Fetalis Including Exchange Transfusion Technic Boston 1957 1958 Little Brown & Co
2 Allen J C Emerson D M Darron E S G and Sykes C Pooled Plasma With Little or No Risk of Homologous Serum Jaundice J A M A 154 103 1954
3 Arthurton M O Brien D and Mann T Haemoglobin Levels in Premature Infants Arch Dis Childhood 29 38 1954
4 Birkhill F R Maloney M A and Levenson S M Effect of Transfusion Polycythemia Upon Bone Marrow Activity and Erythrocyte Survival in Man Blood 6 1021 1951
5 Cathie I A B The Treatment of Erythroblastosis Fetalis Arch Dis Childhood 21 229 1946
6 Dacie J V Transfusion of Saline Washed Red Cells in Nocturnal Haemoglobinuria (Marchiafava Micheli Disease) Clin Sc 7 65 1948
7 Donegan C C Jr MacIlwaine W A and Leavell B S Hematologic Studies on Patients With Sickle Cell Anemia Following Multiple Transfusions Am J Med 17 29 1954
8 Dubin I N Idiopathic Hemochromatosis and Transfusion Siderosis a Review Am J Clin Path 25 514 1955
9 Ellis J T Schulman I and Smith C H Generalized Siderosis With Fibrosis of Liver and Pancreas in Cooley s (Mediterranean) Anemia With Observations on the Pathogenesis of the Siderosis and Fibrosis Am J Path 30 287 1954
10 Elmlinger I J Huff R L and Oda J M Depression of Red Cell Iron Turnover by Transfusion Proc Soc Exper Biol & Med 79 16 1952
11 Forum The Anemia of Prematurity Should It Be Treated by Transfusion? Quart Rev Pediat 8 1 1953
12 McGovern J J Platelet Transfusions in Pediatrics New England J Med 256 922 1957
13 Miller G McCoord A B Joos H A and Clausen S W Studies of Serum Electrolyte Changes During Exchange Transfusion Pediatrics 13 412 1954
14 Mollison P L Blood Transfusion in Clinical Medicine ed 2 Springfield Ill 1956 Charles C Thomas Publisher
15 Penick G D and Brinkhous K M Relative Stability of Plasma Antihemophilic Factor (AHF) Under Different Conditions of Storage Am J M Sc 232 434 1956
16 Pickles M M Haemolytic Disease of the Newborn Springfield Ill 1949 Charles C Thomas Publisher
17 Robertson O H The Effects of Experimental Plethora on Blood Production J Exper Med 26 221 1917
18 Schulman I Smith C H Stern G S Studies on the Anemia of Prematurity A M A Am J Dis Child 88 567 1954
19 Smith C H Schulman I Ando R E and Stern G Studies in Mediterranean (Cooley s) Anemia I Clinical and Hematologic Aspects of Splenectomy With Special Reference to Fetal Hemoglobin Synthesis Blood 10 582 1955
20 Smith C H Schulman I Ando R E and Stern G Studies in Mediterranean (Cooley s) Anemia II The Suppression of Hematopoiesis by Transfusions Blood 10 707 1955
21 Stefanini M and Dameshek W Collection Preservation and Transfusion of Platelets With Special Reference to the Factors Affecting the "Survival Rate" and the Clinical Effectiveness of Transfused Platelets New England J Med 248 797 1953
22 Strumia M M The Preservation of Blood for Transfusion Blood 9 1105 1954
23 Wiener A S Prevention of Accidents in Blood Transfusions J A M A 156 1301 1954

serum) generally between the first and second weeks of life Immaturity of the liver previously held responsible for the failure to excrete bilirubin resulting from normal or slightly accelerated red cell destruction[6] now finds more precise definition in the delayed development of an enzyme system required for the conversion of indirect to direct bilirubin

Mechanical fragility of the red cells in the first days of life indicates their increased susceptibility to hemolysis[22] On the other hand two studies relating to the transfusion of placental blood into another infant or an adult[29] indicate that fetal red cells have only a somewhat shorter survival than those of the adult Only the red cells produced during late fetal life survive for a shorter time than expected

The serum bilirubin of the full term infant seldom exceeds 10 mg per 100 ml of serum at its height on the second and third days of life and usually reaches no more than 7 mg per 100 ml In the premature infant unaffected by erthyroblastosis there is a tendency toward excessive bilirubinemia[31 46] as compared with the full term infant The bilirubin level follows that of the full term infant in the first two days of life but then continues its rise to the fourth and sometimes fifth day before a decline occurs The premature infant tends therefore to accumulate bilirubin beyond the two day period of the full term baby Peak levels in the premature infant average 12 mg[31] and may exceed 20 mg per 100 ml of serum[10] According to some authors hyperbilirubinemia exists in the premature infant if a level of more than 10 to 15 mg per 100 ml occurs during the first week of life Physiologic jaundice is present in infants with lower levels[37]

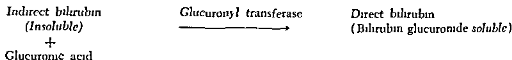
Jaundice in the first twenty four hours of life is never physiologic In infants with erythroblastosis in contrast to those with physiologic icterus jaundice develops rapidly with high bilirubin levels on the first day of life and maximum concentrations often reached in less than two days In the absence of isoimmunization excessive bilirubin levels in the full term or premature infant develop more slowly and usually present initial problems of management beyond the first forty eight hours of life

Hyperbilirubinemia of the Newborn Infant Unrelated to Isoimmunization Hyperbilirubinemia unrelated to erythroblastosis may reach a magnitude in the full term and the premature infant sufficient to place the infant in jeopardy of brain damage In a series of such infants bilirubin levels (indirect reacting) ranged from 12 mg per 100 ml on the first day of life to 20 mg and above (maximum 32 mg) during the ensuing week[1] The peak level was reached earlier in the week in the full term infant than in the premature infant The degree of hyperbilirubinemia is regarded as an accentuation of the mechanism involved in the pathogenesis of physiologic jaundice The premature infant particularly requires careful watching because of the tendency toward excessive bilirubinemia and subsequent kernicterus

In this group of patients with nonerythroblastotic hyperbilirubinemia there are conflicting views as to the critical levels for exchange transfusion or even for its routine use The low incidence of kernicterus in untreated premature infants (less than 1 per cent) led one group of investigators to conclude that preventive treatment of kernicterus should be on an individual rather than a routine basis[44] This was based on the fact that the existence of a critical peak concentration of

obstructive and hepatogenous jaundice in whom the plasma contains largely conjugated bilirubin (water soluble form) giving a predominantly direct van den Bergh reaction bilirubinuria is present

Enzymatic Conversion of Indirect to Direct Bilirubin Indirect bilirubin formed by the reticuloendothelial cells is transported in the plasma attached to proteins mainly albumin [3] There is evidence[7,8] that the excretion of water insoluble free indirect bilirubin *depends upon its conversion to a direct reacting* water soluble pigment and that the main pathway is through conjugation with glucuronic acid It has also been demonstrated that the formation of bilirubin glucuronide in liver depends upon the transfer by an enzyme in liver microsomes *(transferase) of glucuronic acid from uridine diphosphate glucuronic acid* Free glucuronic acid however does not enter into the conjugation of indirect bilirubin This conversion may be schematically represented as follows



According to this concept before birth the placenta and not the liver is responsible for removal of bilirubin from the fetal blood [4] The infant therefore is not born jaundiced and only becomes so if the enzyme system responsible for the conjugation of bilirubin has not yet been fully developed It may be assumed that the fall in pigment concentration during the first week of life depends upon a corresponding rise in bilirubin transferase activity toward adult levels [8]

The effect of this enzyme deficiency is manifested particularly in patients with erythroblastosis as a result of which the newborn infant is exposed to high plasma concentrations of bilirubin Excessive hyperbilirubinemia occurring occasionally in the full term and premature infant in connection with physiologic jaundice and unrelated to blood group incompatibility is based on the same difficulty of converting indirect to direct bilirubin [10] An unidentified substance in the plasma of the pregnant woman which inhibits the in vitro conversion of indirect bilirubin to direct bilirubin has been demonstrated by rat liver slices This inhibitor may conceivably contribute to the development of hyperbilirubinemia in the newborn *infant* [36a]

Conditions such as Gilbert's disease [8] familial nonhemolytic jaundice [19] and the Crigler Najjar syndrome [1] in which the jaundice is due to the accumulation of indirect (nonconjugated) bilirubin may now be explained as a failure *of the liver to form* direct bilirubin (bilirubin glucuronide) because of a deficiency of the specific enzyme transferase

JAUNDICE IN THE EARLY NEONATAL PERIOD

Physiologic Jaundice (Icterus Neonatorum) Jaundice appears commonly in newborn infants from the second to the fifth day of life After reaching a maximum intensity between the second and third days the jaundice begins to diminish with bilirubin dropping to normal levels (0 25 to 0 75 mg per 100 ml of

Hereditary Nonspherocytic Hemolytic Disease Hereditary nonspherocytic hemolytic disease may also present itself at birth with the clinical picture of erythroblastosis[11] Absence of spherocytosis normal osmotic fragility and failure to respond to splenectomy rule out hereditary spherocytosis A negative Coombs test and the absence of Rh incompatibility eliminate erythroblastosis and persistence of the jaundice rules out ABO involvement

Elliptocytic (Ovalocytic) Hemolytic Anemia Elliptocytic (ovalocytic) hemolytic anemia associated with jaundice on the second day of life has been reported but anemia was not noted until the infant was 1 month of age[37]

Heinz Body Anemia in the Newborn Infant Heinz body anemia in the newborn infant has been described as a hemolytic anemia occurring in premature infants[6] These babies develop a nonobstructive jaundice in the first few days of life usually between the first and fifth days Anemia appears in the second to the third week as the jaundice fades The Coombs test is negative Examination of the blood before the development of the anemia shows a progressive increase in the number of Heinz bodies (9 to 45 per cent of the red cells) Originally described in premature and congenitally defective infants Heinz body anemia has also been reported in full term infants[61]

Heinz bodies are refractible intracellular inclusions in the red cells which are readily seen in fresh wet preparations or in preparations with supravital dyes such as brilliant cresyl blue They are composed of denatured globin and serve as an indication of a hemolytic process The blood smear shows anisocytosis target cells irregularly shaped fragmenting cells microspherocytes and basophilic stippling

The disease is self limited provided the child survives the original hemolytic episode No evidence of exogenous toxic substances are found before during or after birth High dosages of vitamin K (Synkavite) have been incriminated as a possible cause of Heinz body anemia since severe hemolytic anemia with some Heinz body formation can be produced experimentally in animals by administration of relatively large doses of Synkavite[4] Another possibility is the existence of an inherent defect in red cells rendering them susceptible to hemolysis analogous to primaquine sensitivity in which Heinz bodies also occur

Acute Hemolytic Anemia Related to Naphthalene The ingestion of naphthalene (moth balls and flakes) has been described in children as a cause of severe illness with fever nausea diarrhea and evidences of an acute hemolytic anemia The initial cases were described in four Negro infants[6] Fatal naphthalene intoxication resulting from absorption through the skin from diapers stored in moth balls and crystals has been reported in newborn infants[51] Profound anemia hemoglobinuria hemoglobinemia normoblastemia methemoglobinemia leukocytosis jaundice cyanosis spherocytosis occasional Heinz bodies and hemoglobinuria are noted Whole blood or packed cells are essential in treatment[15]

As is true of primaquine sensitivity the susceptibility to naphthalene poisoning depends upon an intrinsic and genetically determined defect of the red cells which renders them especially susceptible to hemolysis (see Chapter 15) The abnormality is in glutathione metabolism which expresses itself in the older erythrocytes As stated elsewhere the basic biochemical defect is a deficiency

plasma bilirubin in premature infants above which kernicterus could be expected to develop could not be demonstrated in their series. Nevertheless levels of 20 mg. and more of indirect bilirubin serve as well established guide posts for orientation and management in this group as in that with erythroblastosis. The treatment of this type of hyperbilirubinemia will be discussed further in Chapter 10.

Relation of Vitamin K to Hyperbilirubinemia, Kernicterus, and Hemolytic Anemia. Large intramuscular doses of a vitamin K preparation (Synkavite) have been shown to increase the serum bilirubin levels in infants during the first week of life.[43] The most severe bilirubinemia, hence the risk of kernicterus, tends to occur in the premature infant in whom elevated bilirubin levels are normally prone to exist.[9] Large dosages of vitamin K are also hepatotoxic, hemolytic, or both. The liberation of excessive amounts of bilirubin resulting from increased hemolysis in the newborn premature infant following excessive vitamin K dosage has been demonstrated.[3] Because of these possibilities it is unwise to exceed stated dosages for the prophylaxis and treatment of hemorrhagic disease of the newborn infant. There is ample evidence that a single dose of a water soluble analogue equivalent to 1 mg. of synthetic vitamin K (menadione) is adequate to prevent hemorrhagic disease in the newborn infant. This would correspond to 3 mg. of menadione sodium diphosphate (Synkavite) vitamin K analogue.[1]

It has been observed[18] that the parenteral administration of a large amount of a vitamin K analogue (Hykinone) to mothers during labor resulted in marked early hyperbilirubinemia in newborn premature infants which caused central nervous system involvement in several cases.

The hemolytic effects of vitamin K have been explained on the basis of an inherent glutathione instability exhibited by the red cells of newborn infants. Incubation of the red cells of newborn infants with vitamin K analogues results in a characteristic fall in glutathione content which accounts for their in vivo hemolysis.[6a] This phenomenon corresponds with the susceptibility to hemolysis of selected persons who are sensitive to primaquine and naphthalene.

Hereditary Spherocytosis. Hereditary spherocytosis is manifested occasionally in the newborn period,[19, 3] at which time it may be confused with erythroblastosis. Microspherocytosis is present in patients with both hereditary spherocytosis and ABO (not Rh) erythroblastosis. In those with erythroblastosis the microspherocytosis lasts a few days, whereas it persists in those with hereditary spherocytosis. Incompatibility between the blood groups of the mother and fetus and frequently a positive direct and indirect Coombs test characterize erythroblastosis. In patients with hereditary spherocytosis the Coombs test is uniformly negative, a positive family history is common, and an increased erythrocyte fragility is noted in the blood of affected persons.

Exchange transfusions to prevent kernicterus are indicated for the treatment of patients with hyperbilirubinemia as in those with erythroblastosis. It is as important to remove congenitally defective and readily destructible red cells from the blood of patients with spherocytic anemia as it is to remove sensitized cells from the blood of those with erythroblastosis.

pain fatigue dark urine and slight enlargement and tenderness of the liver The direct bilirubin accounts for about 60 per cent of the total serum bilirubin The liver cell is apparently able to conjugate indirect bilirubin with glucuronic acid and convert it to direct bilirubin but cannot excrete it into the bile The most striking finding in all cases is the presence of a coarsely granular brown pigment in a sharp centrolobular distribution in the parenchymal cells A familial history of jaundice has been occasionally obtained in siblings [35] This type of bilirubinemia in which the direct bilirubin fraction predominates is to be differentiated from Gilbert's disease [9] familial nonhemolytic jaundice of Dameshek and Singer [18] and the Crigler Najjar syndrome[17] in which the indirect reacting bilirubin is in excess Although the Dubin Johnson syndrome has been observed principally in adults it has been recorded occasionally in infancy but not specifically in the early or late neonatal period

We have recently encountered an infant jaundiced from birth with blood groups compatible with those of the mother who probably had a variant of this disease The Coombs test was negative In the first two weeks of life the total bilirubin ranged from 47 to 59 mg per cent with a direct fraction of 33 to 40 mg and an indirect fraction of 13 to 19 mg Although the Coombs test was negative the infant was given an exchange transfusion on the third day of life because of the mounting indirect fraction The mother her sister and her two brothers have been chronically jaundiced but in good health Their total bilirubin ranged from 6 to 13 mg per cent of which the direct reacting fraction ranged from 4 to 11 mg and the indirect from 1 3 to 4 1 mg per cent Liver biopsy in the mother however showed no pathognomonic pigmentation of the parenchymal cells as described in the Dubin Johnson syndrome The infant's jaundice and bilirubinemia receded over the period of several months

A closely allied disease but without pigment changes in the liver is that described by Rotor [60] This too is a familial type of nonhemolytic jaundice characterized by a direct van den Bergh reaction The case described may fall into this category

JAUNDICE IN THE LATER NEONATAL PERIOD

Prolonged Obstructive Jaundice Several diseases of early infancy are characterized by evidence of biliary obstruction which finds its inception in the first weeks of life Patients with diseases associated with biliary obstruction and those with absence of the bile ducts have been designated as members of a group with prolonged obstructive jaundice Individual diagnosis is usually difficult but complexities are resolved at times by scrupulous and continuous appraisal of clinical and laboratory findings

This group of cases has been classified by Hsia and co workers[34] into the following categories biliary obstruction caused by inspissation of bile either from hemolysis as occurs in erythroblastosis or of multiple etiologies and congenital atresia of the intrahepatic and extrahepatic ducts including the common bile duct Neonatal hepatitis has been included as one of the causative factors in the inspissated bile syndrome of nonerythroblastotic origin

Prolonged obstructive jaundice is characterized by jaundice increasing after the first week of life but varying in its intensity after a maximum has been reached Additional features are light stools dark urine elevation of both total and direct serum bilirubin levels decreased urobilinogen in the urine and stool and moderate to marked enlargement of the liver and spleen Whereas the level

of glucose 6 phosphate dehydrogenase This enzyme is essential in maintaining glutathione in a reduced state which is necessary to protect intracellular hemoglobin from the toxic effects of a number of drugs *These defects were described* in two newborn infants with hemolytic anemia who had been exposed to articles of clothing impregnated with moth balls [19] In another case [60] naphthalene and its metabolites passed through the placenta to the newborn infant producing jaundice and evidences of a hemolytic anemia The mother who had ingested moth balls had a profound hemolytic anemia An exchange transfusion in the infant was performed when the indirect bilirubin rose to nearly 20 mg per 100 ml from which point the course was uneventful

Infections in Newborn Infants Icterus associated with congenital syphilis sepsis [6] toxoplasmosis and cytomegalic inclusion disease may result from excessive blood destruction as well as from liver cell damage Pneumonitis congenital heart *disease meningitis urinary tract infection and intracranial hemor*rhage are some of the pathologic states associated with hyperbilirubinemia The elevation of both the direct and indirect serum bilirubin levels in patients with these conditions reflects excessive hemolysis of red cells as well as hepatocellular damage [7]

Cytomegalic Inclusion Disease Cytomegalic inclusion disease which may appear at or shortly after birth should be suspected especially in the prematurely born infant in the presence of jaundice purpura hepatosplenomegaly and neurologic disturbances [4] Other features include hemolytic anemia, thrombocytopenia petechiae hematuria erythroblastemia pneumonitis and cerebral calcification

The disease is characterized pathologically by an *inclusion containing* giant cell appearing predominantly in the lining of the ducts of various organs such as the alveoli of the lungs tubular epithelium of the kidney and the brain The diagnostic cytomegalic or owl eyed cells with the large intracytoplasmic or intranuclear inclusion bodies are found in the sediment of urine kept under refrigeration[40] and in gastric washings [4] The infectious agent has been ascribed to a species specific salivary gland virus Cytopathogenicity of this virus obtained from the mouth and urine for tissue cultures of human fibroblasts provides a means for study of the human infection with this virus [38]

Congenital Toxoplasmosis Congenital toxoplasmosis is a rare cause of neonatal jaundice but is probably more frequent than is suspected This disease may be confused at the onset with erythroblastosis *fetalis* [6] Congenital toxoplasmosis may be associated with deep jaundice and in a recently observed infant the serum bilirubin totaled 16 mg per 100 ml of which 13 mg was of the indirect type A normal hemoglobin level a negative Coombs test and absence of *intragroup incompatibility* help to differentiate this condition from erythroblastosis In the active disease the parasites have been demonstrated in smears of the spinal fluid The diagnosis can also be confirmed by serologic tests of various types [4]

Chronic Idiopathic Jaundice (Dubin Johnson Type Dubin Sprinz Disease) Chronic idiopathic jaundice of Dubin Johnson type [3, 17] manifests itself as a form of chronic or intermittent jaundice of fluctuating intensity with abdominal

pain fatigue dark urine and slight enlargement and tenderness of the liver The direct bilirubin accounts for about 60 per cent of the total serum bilirubin The liver cell is apparently able to conjugate indirect bilirubin with glucuronic acid and convert it to direct bilirubin but cannot excrete it into the bile The most striking finding in all cases is the presence of a coarsely granular brown pigment in a sharp centrolobular distribution in the parenchymal cells A familial history of jaundice has been occasionally obtained in siblings[3] This type of bilirubinemia in which the direct bilirubin fraction predominates is to be differentiated from Gilbert's disease [5] familial nonhemolytic jaundice of Dameshek and Singer[18] and the Crigler Najjar syndrome[17] in which the indirect reacting bilirubin is in excess Although the Dubin Johnson syndrome has been observed principally in adults it has been recorded occasionally in infancy but not specifically in the early or late neonatal period

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of serum bilirubin remains relatively constant in infants with biliary atresia it may fluctuate in those with the other groups of disease Extensive erythropoiesis is found in the liver of patients with the inspissated bile syndrome due to erythroblastosis but none is found in patients with the other syndromes The liver flocculation tests for liver function are negative but may be transiently positive No single laboratory test can be used for specific differentiation Individualization is necessary and the course in each patient must be followed clinically and with a combination of diagnostic laboratory tests

Obstructive Jaundice Complicating Erythroblastosis—Inspissated Bile Syndrome The jaundice in most infants with erythroblastosis fetalis begins to subside by the fourth or fifth day and terminates by the end of ten days Occasionally the jaundice persists the stools become pale and bile appears in the urine the entire picture closely simulating congenital biliary atresia The obstructive phenomena may be of short or long duration When prolonged it acquires the clinical and pathologic characteristics of the inspissated bile syndrome although qualitatively the basic features are similar regardless of duration

Obstructive jaundice may occur at the height of the hemolytic process in erythroblastosis or not until some weeks later Occasionally an evanescent phase of hemolytic jaundice occurs soon after birth with a recurrence of the jaundice associated with biliary obstruction after a few weeks[4] When obstruction appears in the first days of life the total serum bilirubin becomes progressively elevated mainly due to the direct fraction and then recedes slowly to normal levels within a few weeks When it persists at intermediate levels for prolonged periods it is categorized as obstructive jaundice due to the inspissated bile syndrome

Exchange transfusions have a negligible effect on the percentage of direct bilirubin and do not alter the progression of biliary obstruction[59] This procedure should be carried out however if there is a significant elevation of indirect reacting pigment which in contrast to direct reacting bilirubin carries the hazard of cerebral damage In the reported cases[59] the elevated direct serum bilirubin level decreased in most patients spontaneously from eighteen hours to six days It is of interest that the levels of direct bilirubin in the cord blood were higher than those in normal blood (approximately 1 mg per 100 ml)

In a newborn infant under our observation the cord blood bilirubin rose from a total of 17 to 48 mg with 12 to 35 mg direct reacting and 5 to 13 mg indirect reacting pigment by the fourth day of life The next day this value was lower and continued to decrease during the following week although normal blood levels were not achieved until the second month In another infant who was not seen until the fifth day of life liver involvement was apparent when the total bilirubin on that day measured 43 mg of which 31 mg was direct and 12 mg indirect On the eleventh day the total bilirubin was 121 mg of which 83 mg was the direct fraction and 38 mg indirect Jaundice then began to recede and by the third week the total bilirubin measured 17 mg per cent the direct fraction 14 mg and indirect 3 mg In the ninth week there was a total of 7 mg with 6 mg indirect Except for a slight transient elevation of thymol turbidity in the second month there was no evidence of liver involvement At 13 months of age the infant was still normal without signs of nervous system involvement

The degree of biliary obstruction is usually partial—rarely complete Jaundice occurs between 3 weeks and 6 months of age with an average duration of seven to eight weeks [31] In cases of prolonged jaundice extending into the second month there is a progressive increase of direct as well as indirect bilirubin The precise reason for the inspissated bile syndrome complicating erythroblastosis is unknown whether it be due to hepatocellular [9] damage incurred by the hemolytic disease or to the overload of pigment causing stasis and complete block Multinucleated giant liver cells found in neonatal hepatitis have also been described in this condition Recovery has been ascribed to cessation of the hemolytic process and enlargement of biliary ducts with increasing age [34]

In differentiating the inspissated bile syndrome from other categories of obstructive jaundice the history clinical observation and laboratory tests are helpful Patients with the inspissated bile syndrome due to erythroblastosis demonstrate the following clinical and laboratory features clinical evidence of erythroblastosis during the early acute phase jaundice on the first day of life which persists longer than the first three weeks an obstructive type of jaundice with marked elevation in both direct and indirect bilirubin levels negative liver flocculation tests and demonstration of maternal fetal blood group incompatibilities [34] Spontaneous recovery is the rule in patients with obstructive jaundice in this category

Inspissated Bile Syndrome of Unknown Etiology No single factor has been established for the etiology of the nonerythroblastotic type of the inspissated bile syndrome and the exact limits of this classification have not been defined Neonatal hepatitis has long been regarded as the basic pathology but no unequivocal evidence has been offered for its causation by the agents of homologous serum hepatitis or the virus of infectious hepatitis [3] Focal or widespread inflammatory degeneration is found in the liver with formation of multinucleated giant hepatic cells [16] This morphologic change has been regarded however as the major abnormality rather than as a nonspecific finding in this syndrome Plugging by inspissated bile in bilary canaliculi is of secondary importance [9] This giant cell transformation has also been observed however with intrahepatic and extrahepatic abnormalities and has led to the hypothesis that neonatal hepatitis represents a development defect of liver cells resulting in aplasia of the bile capillaries and malformation of liver cells [5] The relation of these morphologic changes to neonatal hepatitis is by no means settled Factors such as small bile ducts dehydration and hepatic immaturity contribute to biliary stasis The recovery of an inclusion cell virus from three infants with neonatal hepatitis [63] suggests such an etiology for a group of cases of this disorder

The urine contains bile and the stools are acholic green or yellow The flocculation tests of liver function are usually negative The direct and total serum bilirubin levels are always elevated and show either a slowly falling or variable trend

Patients in this category are often noted to be poorly nourished as compared with those in other categories who exhibit good health About 60 per cent of these infants recover without sequelae some develop cirrhosis and other die

of surgical complications[3] A recessive mode of inheritance has been suggested in this syndrome[8]

Atresia of the Bile Ducts Congenital atresia of the extrahepatic bile ducts represents about 60 per cent of cases of prolonged obstructive jaundice in infancy[7] Despite persistent and progressive jaundice affected infants appear well nourished in the first three or four months of life The stools are acholic the urine is dark and the level of bilirubin is constant It has been estimated that of about 20 per cent of patients amenable to surgical anastomosis, only 2 to 3 per cent of the total with chronic obstructive jaundice due to atresia of the extrahepatic duct system have been treated successfully[6] The remainder succumb in the immediate postoperative period or later to biliary cirrhosis

Congenital atresia of intrahepatic ducts may occur with or without an associated extrahepatic atresia Diagnosis is only possible by biopsy In general the findings resemble those in patients with congenital atresia of the large extrahepatic bile ducts In a group of patients in whom atresia of both intrahepatic and extrahepatic ducts occurred together the course was more benign with a life span of three to five years[1] It was later demonstrated that the intrahepatic bile ducts are present in the early months of life and disappear coincident with prolonged disturbance in bilirubin elimination through the hepatic cells into the biliary canaliculi giving histologic evidence of cholestasis[18]

Management of Prolonged Obstructive Jaundice Although evaluation from clinical and laboratory sources may be helpful exploratory laparotomy is sometimes undertaken when jaundice is unduly prolonged The problem becomes increasingly perplexing from the second to the fourth months of life when the type of liver involvement is not discernible and the possibility even though vague of biliary atresia is entertained One of the drawbacks to early surgical intervention is the finding that such a procedure in infants under 3 months of age with liver damage is attended with serious risk[4]

Gellis and Hsia[7] have provided indications for surgical exploration that are eminently practical Operation is indicated if there is a consistent absence of bile in the stools and duodenal secretions and of urobilinogen in the urine if the initial bilirubin level is low and shows a slowly progressive increase with no variability in pattern if the flocculation tests are negative and if there is no blood group incompatibility to suggest erythroblastosis Operation should be deferred longer if there is bile in the stools or duodenal secretions even in small amounts if urobilinogen is present in the urine in normal dilutions if the initial bilirubin level is high and has shown a tendency to fall or be highly variable with no definite upward trend if the flocculation tests are positive and if blood group incompatibility suggesting erythroblastosis can be demonstrated

By examining liver tissue in cases of biliary atresia Christy and Boley[14] have found that a large amount of fibrosis was already present within the first two months after birth and that it tended to increase with age Instead of extensive abdominal explorations which are hazardous Swenson and Fisher[1] have described a limited exploratory operation with direct cholangiography utilizing the gall bladder and outlining the extrahepatic duct system with Diodrast A liver biopsy is made at the same time Subsequent extensive exploration

is undertaken if no hepatic or common duct is visualized With this program an exploratory procedure and correction can be undertaken before the third month of life

Medical treatment with magnesium sulfate and other cholagogues to establish an increased flow of bile has been reported[1, 45] but has thus far proved unsuccessful Steroids given orally occasionally will lower the bilirubin level in patients without biliary atresia but experience with their use is still limited

Galactosemia Galactosemia almost always is accompanied by obstructive jaundice during the first week of life and persists for a variable length of time thereafter In the absence of anemia hepatomegaly and a positive reaction for sugar warrant an examination for galactosemia (The tape test does not detect galactose in the urine) The congenital inability to convert galactose to glycogen in the liver is corrected by feeding the infant a milk substitute [6, 36]

Congenital Familial Nonhemolytic Jaundice With Kernicterus Congenital familial nonhemolytic jaundice with kernicterus has been reported by Crigler and Najjar[17] Six of eight infants in a single large family had jaundice with severe neurologic disease from the second day of life until death which usually occurred within the first year of life There was no incompatibility of blood groups or evidence of hemolytic disease The indirect serum bilirubin level was constantly elevated with minimal elevations in the direct fraction The affected infants showed evidence of kernicterus between 2 weeks and 3 months of age

The abnormality depends upon a genetic defect consisting of an inability of hepatic cell to metabolize and excrete bilirubin There is evidence to show that the abnormally high levels of indirect bilirubin represent defective conjugation with glucuronic acid required for conversion to soluble direct bilirubin These patients presumably are deficient in the same bilirubin glucuronyl transferase enzyme that is slow to develop in the newborn infant

Exceptions to the occurrence of kernicterus were seen in two of the children in this family now 2 and 5 years of age who have had persistent jaundice from birth but who have shown no neurologic disability[13] In another case of this disease not in the same family neurologic symptoms did not appear until the patient was 3 years of age[49] despite the persistence of jaundice from birth

Except for the development of kernicterus this condition corresponds to a type of retention jaundice which may be readily confused with chronic hemolytic jaundice—namely *familial nonhemolytic jaundice*[18] It represents a form of constitutional hepatocellular dysfunction resulting in an accumulation of indirect bilirubin

Anemia microcytosis spherocytosis reticulocytosis increased fragility of the red cells splenomegaly and increased excretion of urobilinogen in the urine and stools which characterize congenital spherocytic anemia are absent in this condition Jaundice in this form of familial nonhemolytic jaundice usually appears soon after birth and persists throughout life The concentration of bilirubin in the serum seldom exceeds 5 mg per 100 ml with a range of 12 to 15 mg per cent and is practically all of the indirect type In most instances the disease is found in young adults and follows a benign clinical course without bilirubin encephalopathy The Crigler Najjar syndrome[17] on the other hand is associated

with severe hyperbilirubinemia with 10 to 44 mg of serum bilirubin per 100 ml with the greater portion primarily of the indirect type

Jaundice and Carotenemia Carotenemia occasionally confused with jaundice refers to a harmless yellowish discoloration of the skin which occurs in infants and young children due to increased lipochromes in the blood from ingestion of carrots squash spinach egg yolk and other sources of dietary pigment The discoloration appears especially in the skin of the palms soles forehead and nasolabial folds In contrast to jaundice it is absent from the conjunctivae and buccal mucous membranes The icterus index is elevated but the van den Bergh reaction is negative [1]

Jaundice Due to Pyloric Stenosis Jaundice has been described [5a] with a marked increase in the indirect reacting bilirubin in the serum of patients with pyloric stenosis due to posterior angulation of the pyloric tumor and extrinsic pressure on the common bile duct With operative treatment jaundice disappeared promptly This combination assumes importance only because of the need for differentiation from other contemporaneous types of obstructive jaundice

Jaundice and Hypothyroidism Prolonged physiologic jaundice has been encountered in connection with congenital cretinism This association may lead to an early diagnosis and treatment of the hypothyroid condition which might otherwise not have been suspected

Hematomas Mild jaundice occasionally follows absorption of bilirubin from hematomas in diverse locations including those in intracranial and subdural sites

Miscellaneous In addition to cretinism and pyloric stenosis prolonged physiologic jaundice may be due to other abnormalities such as congenital heart lesions and kidney disease [64]

REFERENCES

1 Ahrens E H Jr Harris R C and MacMahon H E Atresia of the Intrahepatic Bile Ducts Pediatrics 8 628 1951
2 Akerren Y Early Diagnosis and Early Therapy in Congenital Cretinism Arch Dis Childhood 30 254 1955
3 Allison A C Danger of Vitamin K to Newborn Lancet I 669 1955
4 Allison A C Acute Haemolytic Anaemia With Distortion and Fragmentation of Erythrocytes in Children *Brit J Haemat 3 1 1957*
5 Arias I M and London I M Bilirubin Glucuronide Formation in Vitro Demonstration of a Defect in Gilbert s Disease Science 126 563 1957
5a Arias I Schorr J B and Fraad L M Clinical Conference Congenital Hypertrophic Pyloric Stenosis With Jaundice Pediatrics 24 338 1959
6 Bain A D Bowie J H Flint W F Beverley J K A and Beattie C P Congenital Toxoplasmosis Simulating Haemolytic Disease of the Newborn J Obst & Gynaec Brit Emp 63 826 1956
7 Billing B H and Lathe G H The Excretion of Bilirubin as an Ester Glucuronide Giving the Direct van den Bergh Reaction Biochem J 63 6 1956
8 Billing B H and Lathe G H Bilirubin Metabolism in Jaundice Am J Med 24 111 1958
9 Bound J P and Telfer T P Effect of Vitamin K Dosage on Plasma–Bilirubin Levels in Premature Infants Lancet *1 720 1956*
10 Brown A K and Zuelzer W W Studies in Hyperbilirubinemia Hyperbilirubinemia of the Newborn Unrelated to Isoimmunization A M A J Dis Child 93 263 1957

11 Bruton O C Crosby W H and Motulsky A G Hereditary Non spherocytic Hemolytic Anemia Presenting as Hemolytic Disease of the Newborn Infant Pediatrics 13 41 1954
12 Cecil R L and Loeb R F and others (editors) Textbook of Medicine ed 9 Philadelphia 1955 W B Saunders Co p 926
13 Childs B and Najjar V A Familial Non hemolytic Jaundice With Kernicterus a Report of Two Cases Without Neurologic Damage Pediatrics 18 369 1956
14 Christy R A and Boley J O The Relation of Hepatic Fibrosis to Concentration of Bilirubin in the Serum in Congenital Atresia of the Biliary Tract Pediatrics 21 226 1958
15 Cock T C Acute Hemolytic Anemia in the Neonatal Period A M A J Dis Child 94 77 1957
16 Craig J M and Landing B H Form of Hepatitis in Neonatal Period Simulating Biliary Atresia A M A Arch Path 54 321 1954
17 Crigler J F and Najjar V A Congenital Familial Nonhemolytic Jaundice With Kernicterus a New Clinical Entity Pediatrics 10 169 1952
18 Dameshek W and Singer K Familial Nonhemolytic Jaundice Constitutional Hepatic Dysfunction With Indirect van den Bergh Reaction Arch Int Med 67 259 1941
19 Dawson J P Thayer W W and Desforges J F Acute Hemolytic Anemia in the Newborn Infant Due to Naphthalene Poisoning Report of Two Cases With Investigations Into the Mechanism of the Disease Blood 13 1113 1958
20 Dormell G N and Lann S H Galactosemia Report of Four Cases Pediatrics 7 503 1951
21 Doses of Water Soluble Vitamin K Analogues in Hemorrhagic Disease of the Newborn J A M A 164 1331 1957
22 Dubin I N Chronic Idiopathic Jaundice a Review of Fifty Cases Am J Med 24 268 1958
23 Dubin I N and Johnson F B Chronic Idiopathic Jaundice With Unidentified Pigment in Liver Cells a New Clinico pathologic Entity With a Report of 12 Cases Medicine 33 155 1954
24 Eichenwald H F The Laboratory Diagnosis of Toxoplasmosis Ann New York Acad Sc 64 207 1956
24a Findlay L Higgins G and Stanier W Icterus Neonatorum Its Incidence and Cause Arch Dis Childhood 22 65 1947
25 Foulk W T Butt H R Owen C A Jr Whitcomb F F Jr and Mason H L Constitutional Hepatic Dysfunction (Gilbert s Disease) Its Natural History and Related Syndromes Medicine 38 25 1959
26 Gasser C Die hamolytische Fruhgeburtenanamie mit spontaner Innenkorperbildung ein neues Syndrom beobachtet an 14 Fallen Helv paediat acta 8 491 1953
27 Gellis S and Hsia D Y Y Jaundice in Infancy Pediat Clin North America 2 449 1955
28 Gilbert A and Lereboullet P La cholemie simple familiale Semaine méd 21 241 1901
28a Harris R C and Anderson D H Intrahepatic Bile Duct Atresia Paper No 10 American Pediatric Society Swampscott Mass May 5 1960
29 Harris R C Andersen D H and Day R L Obstructive Jaundice in Infants With Normal Biliary Tree Pediatrics 13 293 1954
30 Hollingsworth J W Life Span of Fetal Erthyrocytes J Lab & Clin Med 45 469 1955
31 Hsia D Y Y Allen F H Jr Diamond L K and Gellis S S Serum Bilirubin Levels in the Newborn Infant J Pediat 42 277 1953
32 Hsia D Y Y Boggs J D Driscoll S G and Gellis S S Prolonged Obstructive Jaundice in Infancy the Genetic Components in Neonatal Hepatitis A M A J Dis Child 95 485 1958
33 Hsia D Y Y Goldbloom R B and Gellis S S Studies of the Mechanical Fragility of Erythrocytes Relation to Physiologic Jaundice of the Newborn Infant Pediatrics 13 24 1954

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1 Ahrens E H Jr Harris R C and MacMahon H E Atresia of the Intrahepatic *Bile Ducts Pediatrics 8* 628 1951
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5a Arias I Schorr J B and Fraad L M Clinical Conference Congenital Hypertrophic Pyloric Stenosis With Jaundice Pediatrics 24 338 1959
6 Bain A D Bowie J H Flint W F Beverley J K A and Beattie C P Congenital Toxoplasmosis Simulating Haemolytic Disease of the Newborn J Obst & Gynaec Brit Emp 63 826 1956
7 *Billing B H and Lathe G H The Excretion of Bilirubin as an Ester* Glucuronide Giving the Direct van den Bergh Reaction Biochem J 63 6 1956
8 Billing B H and Lathe G H Bilirubin Metabolism in Jaundice Am J Med 24 111 1958
9 *Bound* J P and Telfer T P Effect of Vitamin K Dosage on Plasma–Bilirubin Levels in Premature Infants Lancet 1 720 1956
10 Brown A K and Zuelzer W W Studies in Hyperbilirubinemia Hyperbilirubinemia of the Newborn Unrelated to Isoimmunization A M A J Dis Child 93 263 1957

With Lipochrome Like Pigment in Liver Cell Report of Four Cases Ann Int Med 41:952 1954

58 Stimey C C and Diamond L K Congenital Hemolytic Anemia in the Newborn Relationship to Kernicterus A M A J Dis Child 94 616 1957

59 Stempfel R Bromian B Exarda F E and Zetterstrom R Obstructive Jaundice Complicating Hemolytic Disease of the Newborn Pediatrics 17 471 1956

60 Swenson O and Fisher J H Surgical Aspects of Liver Disease Pediatrics 16 135 1955

61 Varadi S and Hurworth E Heinz Body Anemia in the Newborn Brit M J 1 315 1957

62 Weech A A Genesis of Physiologic Hyperbilirubinemia In Levine S Z and others (editors) Advances in Pediatrics vol 2 New York 1947 Interscience Publishers Inc p 346

63 Weller T H Macauley J C Craig J M and Wirth P Isolation of Intranuclear Inclusion Producing Agents From Infants With Illnesses Resembling Cytomegalic Inclusion Disease Proc Soc Exper Biol & Med 94 4 1957

64 Gellis S S (editor) Year Book of Pediatrics 1956 1957 Series Chicago 1956 Year Book Publishers Inc p 310

65 Zinkham W H and Child B Effect of Vitamin K and Naphthalene Metabolites on Glutathione Metabolism of Erythrocytes From Normal Newborns and Patients With Naphthalene Hemolytic Anemia A M A J Dis Child 94 420 1957

66 Zinkham W H and Childs B A Defect of Glutathione Metabolism in Erythrocytes From Patients With a Naphthalene Induced Hemolytic Anemia Pediatrics 22 461 1958

67 Zuelzer W W and Apt L Acute Hemolytic Anemia Due to Naphthalene Poisoning J A M A 141 185 1949

34 Hsia D Y Y Patterson J Allen F H Jr Diamond L K and Gellis S S Prolonged Obstructive Jaundice in Infancy General Survey of 156 Cases *Pediatrics 10* 243 1952
35 John G C and Knudtson K P Chronic Idiopathic Jaundice Two Cases Occurring in Siblings With Histochemical Studies Am J Med 21 128 1956
36 Johns D Galactosemia an Unusual Cause of Neonatal Jaundice A M A Am J Dis Child 85 575 1953
36a Lathe G H and Walker M The Inhibitory Effect of Human Pregnancy Serum *Neonatal Serum and Steroids on the Conjugation of Bilirubin by Rat Liver Slices* Biochem J 68 6P 1958
37 Lipton E L Elliptocytosis With Hemolytic Anemia the Effects of Splenectomy J 68 6P 1958
37a Lucey J F Review Article Hyperbilirubinemia of Prematurity Pediatrics 25 690 1960
38 Lucey J F and Dolan R C Injections of a Vitamin K Compound in Mothers and Hyperbilirubinemia in the Newborn Pediatrics 22 605 1958
39 Macaulay D Acholuric Jaundice in a Newborn Infant Arch Dis Childhood 26 241 1951
40 Margileth A M The Diagnosis and Treatment of Generalized Cytomegalic Inclusion Disease of the Newborn Pediatrics 15 270 1955
41 Martin J W and Siebenthal B L Jaundice Due to Hypertrophic Pyloric Stenosis J Pediat 47 95 1955
42 Medearis D N Jr Cytomegalic Inclusion Disease an Analysis of the Clinical Features Based on the Literature and Six Additional Cases Pediatrics 19 467 1957
43 Meyer T C and Angus J The Effect of Large Doses of Synkavit in the Newborn Arch Dis Childhood 31 212 1956
44 Mollison P L Blood Transfusion in Clinical Medicine ed 2 Oxford 1956 Blackwell Scientific Publications p 130
45 McGovern B Resident s Meeting Section on Pediatrics New York Academy of Medicine Dec 13 1951
46 Obrinsky W Allen E L and Anderson D E Physiologic Hyperbilirubinemia in Premature Infants A M A Am J Dis Child 87 305 1954
47 O Donohoe N V Obstructive Jaundice in Haemolytic Disease of the Newborn Treated With Magnesium Sulphate Arch Dis Childhood 30 234 1955
48 Patterson P Study of the Duodenal Fluid in Infants With Jaundice A M A Am J Dis Child 84 415 1952
48a Rapmund G Bowman J M and Harris R C Bilirubinemia in Nonerythroblastotic Premature Infants A M A J Dis Child 99 604 1960
49 Rosenthal I M Zimmerman H J and Hardy N Congenital Non hemolytic Jaundice With Disease of the Central Nervous System Pediatrics 18 378 1956
50 Rotor A B Manahan L and Florentin A Familial Nonhemolytic Jaundice With Direct van den Bergh Reaction Acta med philippines 5 37 1948
51 Schafer W B Acute Hemolytic Anemia Related to Naphthalene Pediatrics 7 172 1951
52 Schmid R Jaundice and Bilirubin Metabolism A M A Arch Int Med 101 669 1958
53 Schmid R Axelrod J Hammaker L and Rosenthal I M Congenital Defects in Bilirubin Metabolism J Clin Invest 36 927 1957
54 Silverman W A Cytomegalic Inclusion Disease Pediatrics 21 682 1958
55 Smetana H F and Johnson F B Neonatal Jaundice With Giant Cell Transformation of Hepatic Parenchyma Am J Path 31 747 1955
55a Smith C H The Magic of Numbers—20 Milligrams of Bilirubin J Pediat 56 712 1960
55b Smith M G The Salivary Gland Viruses of Man and Animals (Cytomegalic Inclusion Disease) Progress in Medical Virology 2 171 1959
56 Smith R T Platou E S and Good R A Septicemia of the Newborn Current Status of the Problem Pediatrics 17 549 1956
57 Sprinz H and Nelson R S Persistent Nonhemolytic Hyperbilirubinemia Associated

subsequent pregnancy with an Rh positive fetus This means that a healthy child usually results from the first pregnancy and that subsequent children are often affected since later pregnancies provide an effective stimulus to an antibody forming mechanism set in motion by the first pregnancy

Clinical Features The diverse symptomatology of erythroblastosis fetalis reflects variations in the degree of red cell destruction blood production and extramedullary hematopoiesis in the liver function In about 15 to 20 per cent of Rh positive infants of Rh negative immunized mothers clinical illness will be absent or hardly perceptible The intensity of the hemolytic process is influenced largely by the titer of maternal anti Rh antibodies A high titer (usually 1 64 and over) or a rising titer carries a serious import and is reflected in the severity of the anemia and depth of jaundice

The clinical findings include jaundice anemia enlargement of the liver and spleen petechiae and in severe cases hemorrhages and edema The liver and spleen may be moderately enlarged at birth or may become palpable as the disease progresses in the first week of life The disease is classified according to increasing severity congenital anemia of the newborn infant the mild form in which pallor becomes apparent as jaundice fades in the first week of life icterus gravis neonatorum in which the infant is more seriously affected with progressive anemia and jaundice and hydrops fetalis (universal edema generalized edema of the newborn infant) the stage in which the stillborn infant is often markedly edematous Except for hydrops fetalis the term erythroblastosis qualified by the degree of severity has supplanted the terminology of the first two categories They represent each end of a continuous range of increasing severity with marked variation in the two salient features of the disease—namely anemia and jaundice

The hydropic infant although usually stillborn and premature may be born alive but seldom survives more than a few hours The massive edema is attributed to heart failure and pulmonary edema Placental excretion of bile pigments in utero supposedly accounts for the lack of jaundice in these infants Mild edema is not necessarily fatal since survival is possible with active treatment

In the infant with erythroblastosis jaundice of the skin and sclerae is almost never present at birth but appears in the first hours of life Although the severity of the hemolytic process cannot be predicted accurately by examination of the amniotic fluid the latter is often pale yellow or yellowish brown and the vernix normal or dark yellow In the latter case the skin underlying the discolored vernix is usually not icteric Edema occurs as a complication in the severely anemic infant with congestive heart failure

Detection of Early Jaundice of the Newborn Infant The need for the early detection of jaundice has been emphasized in infants with erythroblastosis not caused by the Rh factor in whom jaundice is not anticipated such as in those with immunization by A B and rare blood factors In the newborn infant jaundice is generally not apparent until the blood level reaches 4 to 6 mg per 100 ml The absence of jaundice at lower levels may be due to differences in capillary permeability in the newborn baby as compared with the adult or may be due

CHAPTER 9

Erythroblastosis Fetalis (Hemolytic Anemia of the Newborn Infant)—General Considerations

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Definition Erythroblastosis fetalis is a disease of the fetus and newborn infant characterized by a hemolytic anemia based on incompatibility between the blood group of the mother and that of her offspring The disease is designated as such because of the frequent appearance of nucleated red cells in the peripheral blood resulting from their active proliferation in the liver spleen and bone marrow as a compensation for excessive hemolysis

Pathogenesis Isoimmunization results from the presence of an antigenic substance in the fetal red blood cell that is not present in the mother's red blood cells Entrance of this antigenic factor into the maternal circulation either by direct transfusion or by transfer across the placenta from the fetal circulation results in the elaboration of a specific antibody by the mother The transplacental transfer of red cells or antigenic substance is facilitated by the existence in man of a single syncytial cell layer separating the villi containing fetal blood vessels and the maternal sinuses by the thinning out of the expanding placental surfaces and by small leaks in the placental barrier which permit leakage of fetal blood

Sensitization during pregnancies occurs classically when fetal Rh positive blood enters the circulation of the Rh negative mother A similar mechanism is postulated with respect to the ABO groups in heterospecific pregnancies Maternal antibodies recross the placenta gaining access to the fetal circulation where they become attached to fetal red cells resulting in their removal from the circulation and subsequent destruction The various pathologic clinical and hematologic manifestations of erythroblastosis stem from the reaction of these antibodies with fetal red cells in utero and in the neonatal period It has been estimated that only minute amounts of fetal blood (0 03 to 0 07 ml) are required for immunization [3]

A mother sensitized by previous transfusions is likely to have an infant with erythroblastosis in her first pregnancy whereas the first born of a mother immunized during pregnancy is usually free of the disease In the latter situation anti Rh antibodies cannot be detected in the maternal circulation until there is a

subsequent pregnancy with an Rh positive fetus This means that a healthy child usually results from the first pregnancy and that subsequent children are often affected since later pregnancies provide an effective stimulus to an antibody forming mechanism set in motion by the first pregnancy

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to a diminished affinity to pigment in the skin of newborn infants.[19] To safeguard those in whom jaundice is not anticipated, it is suggested that all infants should be inspected frequently during the first two days of life. As aids for the detection of jaundice Allen[2] advises artificial lighting of a specific type and the use of a polished piece of Lucite for blanching the skin.

Kernicterus. Brain damage (kernicterus) represents the most serious complication of erythroblastosis and is associated with high concentrations of indirect bilirubin *from any cause occurring in the first two weeks of life.* *The extent of* potential bilirubin accumulation which may be involved in erythroblastosis can be estimated. Each gram of hemoglobin constitutes a potential source of 35 mg. of bilirubin. For an infant weighing 3 kg., a decrease of 1 gm. of hemoglobin per 100 ml. of blood represents the destruction of 3 gm. of hemoglobin and the source of 105 mg. of bilirubin. A newborn infant handicapped by immature liver enzyme systems therefore may accumulate toxic amounts of bilirubin without evidence of marked anemia.

Kernicterus thus can be regarded as a bilirubin encephalopathy. Cerebral involvement should be suspected in an infant thirty-six hours or more after birth *who feeds poorly, becomes lethargic, and develops muscular twitching or whose* cry is sharp and high pitched. Alteration of the Moro reflex displayed in response to jarring of the crib or a loud noise from the normal embrace reflex of the arms with fists clenched in the affected infant is significant.[3] *Opisthotonus* and respiratory failure follow in the severe and fatal cases. Signs of nervous system involvement should be sought in severely affected full-term infants in whom jaundice reaches a peak intensity at thirty-six hours and in severely affected premature infants in whom jaundice reaches a peak intensity by the fifth or sixth day.

Kernicterus has been established as a postnatal complication and is almost *completely preventable. Although there is a variation in susceptibility, the* likelihood of kernicterus in the infant with erythroblastosis when indirect bilirubin concentrations reach 20 mg. per cent and above has been well documented. Signs of neurologic damage appear thirty-six hours or more after birth.

Assiduous attention to maintaining serum bilirubin concentrations at levels below 20 mg. per cent by one or more exchange transfusions has led to a remarkable reduction in the incidence of kernicterus. Since kernicterus is correlated with the intensity of bilirubinemia, it is important to observe closely those infants who are severely affected clinically at birth and whose cord blood shows elevated bilirubin levels and lowered hemoglobin content. This is especially important in immature infants and in male infants with a high maternal antibody titer since they have been shown to be especially susceptible.

Staining of the basal ganglia not associated with isoimmunization has been described in connection with prematurity,[1, 10, 18] in patients with spherocytic anemia,[40] in those with congenital familial nonhemolytic jaundice,[9] *in newborn* infants given large doses of vitamin K,[4, 8] in premature infants given sulfisoxazole (Gantrisin) in the first five days of life,[151, 39] and even in the newborn infant with severe physiologic jaundice. The use of sulfonamides and salicylates may be hazardous in the newborn infant with hyperbilirubinemia because of the

ability of these substances to displace serum bilirubin from protein binding sites on serum protein and therefore allow its diffusion to other body compartments[33,34]

Kernicterus identical with that seen in human beings occurs naturally in a strain of jaundiced rats[18] The defect is the same in both species namely a lack of the conjugating glucuronyl transferase enzyme in the liver In the animals as in human beings the administration of sulfonamides results in a lowering of the serum bilirubin and intensification of nervous signs and nuclear staining Here too the suggested explanation is that kernicterus results from the competition between bilirubin and the sulfonamides for binding sites on the serum albumin so that unbound bilirubin becomes available for damage to nerve tissue

Since the red cells of the premature infant may be susceptible to hemolysis with the development of jaundice in the infant drug therapy such as excessive doses of vitamin K and sulfonamides should be avoided unless a specific need exists[4]

It has been estimated that approximately 5 to 15 per cent of all live born infants with erythroblastosis will develop kernicterus if untreated About 70 per cent of babies with kernicterus will succumb within seven days of birth Of the 30 per cent that survive the acute stage of cerebral symptoms neurologic sequelae are likely to develop at a later date They constitute about 10 per cent of all cases of cerebral palsy[0] More specifically a fatal outcome is to be expected when signs of cerebral involvement develop on the second day and survival is more common when such signs appear on the fifth day[16] In the group that survive evidences of cerebral damage so frequently present consist of mild to severe spasticity athetosis auditory disturbances and defects of ocular movements[4]

Intense jaundice without signs of kernicterus may be followed by slight mental impairment amounting to a reduction of the intelligence quotient of about 12 per cent[17] Deafness is a common sequel of kernicterus and should be investigated in all affected infants despite the absence of other signs of damage to the nervous system

Children who have recovered from hemolytic disease may show green pigmentation of the deciduous teeth It is of interest that an acute enamel hypoplasia of the deciduous teeth has been observed in patients with kernicterus following erythroblastosis[15]

Pathology The pathologic changes reflect a number of factors duration and intensity of the destruction of the red cells by maternal antibody in utero and in the first days of life compensatory hematopoiesis and effect of abnormal stresses upon the function and structure of immature tissues and organs

In deeply jaundiced infants the entire brain may be pigmented Staining is more intense with a brighter yellow color in the basal ganglia and other nuclei in the brain stem and medulla in the kernicteric infants Ganglion cells degenerate and disappear leaving empty spaces[6] The mechanism of these changes is unknown but it may be related to inhibition by excessive bilirubin of oxygen uptake within the cells[1]

The liver and spleen are invariably enlarged Histologically the most striking feature is widespread extramedullary hematopoiesis in these and other organs such as the pancreas kidneys adrenals lymph nodes thymus and placenta Because of the persistent destruction of red blood cells there is a reversion to the embryonic type of blood formation The bone marrow shows active hematopoiesis with a predominance of immature nucleated red blood cells An enormous proliferation of normoblasts is particularly noticeable within the liver sinusoids compressing and displacing the liver parenchyma cells and resulting in pronounced degenerative changes Many of the hepatic cells are vacuolated and in some cases are replaced by coarsened reticulum Severely damaged livers show evidence of intrahepatic biliary obstruction with masses of bile pigment in the small bile ducts and in biliary canaliculi Hemosiderosis may be found in the spleen and liver Hypertrophy and hyperplasia of the pancreatic islet cells are striking Petechiae even frank hemorrhages may be observed in the lungs The adrenals reveal lipid degeneration of most of the cortical cells [6]

Edema may be localized or severe and generalized as it is in infants with hydrops In this condition extensive edema (especially of the face) varying degrees of maceration effusions into serous cavities and marked enlargement of the spleen and to a lesser degree of the liver are conspicuous findings The placenta in cases of hydrops is edematous bulky and friable The occurrence of erythroblasts in the pulmonary capillaries is the most important single diagnostic sign to be found in the macerated fetus [37]

Maternal Antibodies—Prenatal Testing All pregnant women are initially typed for ABO group and the Rh factor Whether or not the mother is immunized prenatal testing is carried out initially at the first visit or in the first trimester of pregnancy and is repeated after twenty eight to thirty weeks *and at* the end of pregnancy When antibodies develop for the first time they are usually not detectable until about the fourth month of pregnancy They may increase as gestation proceeds and are almost invariably detected by the thirty fifth week in an affected fetus Should the termination of pregnancy be considered before term an additional test may be indicated The absence of anti Rh antibodies in *the mothers serum at this time usually indicates that the infant will not be affected by erythroblastosis due to the Rh factor* [70] *On the other hand* when antibodies are present at the beginning of pregnancy even in weak concentration the birth of an infant with erythroblastosis must be anticipated

Maternal Anti Rh Titer There is some general but not very close correlation between the type and concentration of antibodies and the clinical severity of the disease in the affected child In general the disease tends to be milder when the predominant anti Rh antibodies are of the saline agglutinating type rather than the immune or incomplete form

By and large higher titers of incomplete antibodies are associated with severe disease and a worse prognosis (1 32 to 1 64 and over) Exceptions to severe and fatal cases (stillbirths) with low titers and mild symptoms despite high titers have been noted [46]

Because of the risk of kernicterus *a high maternal anti Rh titer* (1 64 or higher) of incomplete antibodies has been regarded as a sufficient indication for

exchange transfusion immediately after birth in the infant with erythroblastosis regardless of the physical findings or hemoglobin level[43]

A rising anti Rh titer indicates that the fetus is Rh positive The antibodies derived from immunization with a previous Rh positive fetus persist during a subsequent pregnancy with an Rh negative infant (heterozygous father) and have been stated to actually increase in titer[7] This has been challenged by Wiener and co workers[4] who found that pregnancy with an Rh negative fetus and the birth of an Rh negative baby had no effect upon the Rh antibody titer of an Rh negative woman sensitized to the Rh factor

In general if the father is heterozygous and if the maternal antibody titer remains constant the chances are fairly good that the infant will be Rh negative A low titer that fails to change during pregnancy suggests no specific stimulation

There is a great variability in the titer of the sensitized mother following de livery It generally falls somewhat or may continue to be high for prolonged periods

Effect of Previous Transfusions on the Mother Transplacental immunization can be detected in pregnancy when there is a history of blood transfusions or fetal death Transfusions of Rh positive blood or intramuscular injections of Rh positive blood increases an Rh negative mother s susceptibility to immuniza tion by an Rh positive fetus Repeated transfusions are especially potent agents in producing a high degree of sensitization stillbirths and hydrops

Immunization in the Rh Positive Mother and Infant At times there is no obvious incompatibility between mother and child both being Rh positive yet sensitization is indicated by neonatal hemolysis and a positive Coombs test In the absence of ABO incompatibility consideration must be given to immuniza tion with c E Kell and other less frequent factors In such cases the husband s blood also should be examined regardless of his reaction to anti D (Rh) serum for his erythrocytes may possess a minor blood group antigen lacking in those of his wife

Heterozygous and Homozygous Status of the Husband If the husband is Rh positive it is important in management to determine whether he is homozygous or heterozygous for the Rh factor Although this may be reflected to some degree by the presence or absence of the Rh factor in previous offsprings appropriate tests will determine this with greater accuracy The use of anti C anti E anti c and anti e provides a means of determining the husband s probable genotype Since anti d serum is unavailable the diagnosis remains presumptive In the heterozygous father the chances are 50 per cent that the offspring will be Rh positive or Rh negative For the homozygous father (for D or Rh) it can be fairly safely predicted that all the children will be Rh positive

Different Types of Antibodies Antibodies react under varying physical con ditions Some agglutinate red cells suspended in saline solution whereas others will only coat them Both forms are found in the blood of mothers sensitized to Rh positive cells The saline active or "complete" antibodies agglutinate red cells containing the Rh factor they do not cross the placenta and hence are not involved in the causation of erythroblastosis The second type will fail to agglu tinate Rh positive cells when suspended in saline solution but will do so when

The liver and spleen are invariably enlarged Histologically the most striking feature is widespread extramedullary hematopoiesis in these and other organs such as the pancreas kidneys adrenals lymph nodes thymus and placenta Because of the persistent destruction of red blood cells there is a reversion to the embryonic type of blood formation The bone marrow shows active hematopoiesis with a predominance of immature nucleated red blood cells An enormous proliferation of normoblasts is particularly noticeable within the liver sinusoids compressing and displacing the liver parenchyma cells and resulting in pronounced degenerative changes Many of the hepatic cells are vacuolated and in some cases are replaced by coarsened reticulum Severely damaged livers show evidence of intrahepatic biliary obstruction with masses of bile pigment in the small bile ducts and in biliary canaliculi Hemosiderosis may be found in the spleen and liver Hypertrophy and hyperplasia of the pancreatic islet cells are striking Petechiae even frank hemorrhages may be observed in the lungs The adrenals reveal lipid degeneration of most of the cortical cells [6]

Edema may be localized or severe and generalized as it is in infants with hydrops In this condition extensive edema (especially of the face) varying degrees of maceration effusions into serous cavities and marked enlargement of the spleen and to a lesser degree of the liver are conspicuous findings The placenta in cases of hydrops is edematous bulky and friable The occurrence of erythroblasts in the pulmonary capillaries is the most important single diagnostic sign to be found in the macerated fetus [37]

Maternal Antibodies—Prenatal Testing All pregnant women are initially typed for ABO group and the Rh factor Whether or not the mother is immunized prenatal testing is carried out initially at the first visit or in the first trimester of pregnancy and is repeated after twenty eight to thirty weeks and at the end of pregnancy When antibodies develop for the first time they are usually not detectable until about the fourth month of pregnancy They may increase as gestation proceeds and are almost invariably detected by the thirty fifth week in an affected fetus Should the termination of pregnancy be considered before term an additional test may be indicated The absence of anti Rh antibodies in the mother s serum at this time usually indicates that the infant will not be affected by erythroblastosis due to the Rh factor [79] On the other hand when antibodies are present at the beginning of pregnancy even in weak concentration the birth of an infant with erythroblastosis must be anticipated

Maternal Anti Rh Titer There is some general but not very close correlation between the type and concentration of antibodies and the clinical severity of the disease in the affected child In general the disease tends to be milder when the predominant anti Rh antibodies are of the saline agglutinating type rather than the immune or incomplete form

By and large higher titers of incomplete antibodies are associated with severe disease and a worse prognosis (1 32 to 1 64 and over) Exceptions to severe and fatal cases (stillbirths) with low titers and mild symptoms despite high titers have been noted [46]

Because of the risk of kernicterus a high maternal anti Rh titer (1 64 or higher) of incomplete antibodies has been regarded as a sufficient indication for

Except for erythroblastosis due to anti A and anti B when the reaction is often weak or negative a weak reaction due to anti Rh is usually associated with clinically mild forms of the disease

Positive Coombs tests are obtained not only in patients with ABO and Rh erythroblastosis but also in those with idiopathic acquired hemolytic anemia acquired hemolytic anemias secondary to leukemia lymphosarcoma dissem inated lupus erythematosus and thrombotic thrombocytopenic purpura

Indirect Coombs Test The indirect test is used to demonstrate circulating antibodies which coat but do not agglutinate cells *suspended in saline solution* In this in vitro test the serum in question is incubated with corresponding Rh positive red cells of a compatible ABO group Anti globulin serum is added to the incubated red cell suspension which has been thoroughly washed Read ings for agglutination are made as in the direct test If the patient s serum contains incomplete or immune anti Rh antibodies they coat the test red cells and agglu tination follows on the addition of Combs serum The serum can be titrated by preliminary dilution with saline solution and made even more sensitive with the use of trypsinized test cells

Tests With Trypsinized Cells Saline suspended red cells are so altered by preliminary exposure to a trypsin solution as to give direct readings with in complete or immune antibodies[3] This test does not replace the indirect Coombs test for it fails to detect such rare antibodies as Duffy Kell and Kidd factors The method however is widely used and constitutes a valuable adjunct to tests employed for the detection of circulating antibodies

Elution The specificity of the antibody causing damage to the infant s erythro cytes in erythroblastosis can also be demonstrated by elution tests[18, ..., 44] This is a method of releasing antibody from red cells which had adsorbed it The exact nature of the antibody involved may be determined through the use of the heat elution test on umbilical cord blood samples showing a positive Coombs test Eluates are prepared from erythrocytes of umbilical cord blood by prelimi nary washing of the red blood cells in cold buffered saline solution and later sub jecting the suspension to 56 C The eluates are tested for hemagglutination against known A or B red blood cells or positive cells of Rh Hr or other systems The test is particularly valuable when sensitization to antigens in both the Rh and the ABO systems is possible

Frequency of Blood Group Factors Causing Erythroblastosis Until recent years incompatibility with respect to the Rh factor (D) has accounted for the majority of cases of erythroblastosis A lowered incidence however is being noted due to the fact that many parents who have had one or two children with erythroblastosis are limiting the size of their families

Erythroblastosis due to A and B incompatibility is being recognized more widely since there is at present a greater alertness to its presence on the first day of life The frequency of erythroblastosis involving the A and B factors has been estimated as 1 per cent of all newborn infants as compared with 0.5 per cent with the Rh factor (Rh or D)[4] Immunization with A is more frequently encountered than with B

Rh incompatible mating occurs in 13 per cent of all marriages Of incom

these cells are suspended in colloid medium such as plasma serum or albumin These antibodies are variously termed incomplete univalent hyperimmune cryptagglutinoid glutinin immune and albumin Of these incomplete and immune are most commonly used

Blocking" antibodies are a variety of "incomplete agglutinins which fail to agglutinate *test cells* suspended in saline solution or in colloid media They sensitize saline suspended cells by coating their surface The antibody (globulin) coating will be detected with the use of an antihuman globulin (Coombs) serum which agglutinates sensitized cells (direct Coombs test) Many affected Rh positive (D or Rh) infants appear to be Rh negative on typing because the cells are heavily coated with incomplete antibodies The most dependable test to determine the presence of such blocked or coated cells is the application of the direct Coombs test

Naturally occurring antibodies such as anti A and anti B isoagglutinins in persons with group O blood are those normally present throughout life which can be attributed to no known antigenic stimulus Immune anti Rh antibodies are of pathologic and diagnostic significance and originate as a response to a specific stimulus during pregnancy and to transfusion

Transmission of Antibodies Only the incomplete or immune form of anti Rh antibodies are capable of crossing the placenta and this transmission can occur at any early stage of pregnancy When the incomplete and complete forms are present together in maternal serum only the incomplete form appears in the fetal serum[6] While there is some evidence that Rh antibodies are present in human milk and colostrum it is doubtful whether they can be absorbed even in the first days of life[7] Present practice has been to allow mothers to nurse their infants if they want to do so—certainly after the second day Weaning infants with erythroblastosis because maternal milk contains antibodies is not justified

Coombs Test (Antiglobulin Test) The Coombs test is a sensitive method of demonstrating the presence of an antibody adsorbed or bound to red cells This test has come into common use since Coombs and associates[9] found that serum from rabbits immunized with human globulin agglutinated human erythrocytes coated with Rh antibodies Experience has shown this to be an invaluable method of detecting incomplete Rh antibodies The essential component of the Coombs serum *is the anti gamma globulin There are two forms of the Coombs* antihuman globulin test the direct and indirect

Direct Coombs Test The direct test is used to detect antibodies fixed to infants cells in vivo Antiglobulin serum is added to a suspension of cells which has been washed three times with saline solution After the mixture is centrifuged it is observed for agglutination Cells of the cord blood following delivery of an infant to an immunized woman are commonly used in the test to determine coating *or sensitization* while in utero It is useful in confirmation of the typing of an infant presumably Rh negative A positive Coombs test on such blood indicates that the red cells have been sensitized and that the infant is Rh positive The earlier negative test was due to the blocking action of maternal antibody A positive Coombs test establishes *the diagnosis of erythroblastosis but is not in itself* an indication for exchange transfusion

moderately and severely involved infants to be followed by somewhat more severely affected infants[79] Since a woman who has had one stillbirth on an Rh basis has about 4 chances in 5 of losing her next Rh positive fetus[79] it would seem reasonable to advise premature induction of labor if the husband is homozygous

Laboratory Findings—Blood The blood findings include the following features

Red Blood Cells The peripheral blood gives striking evidence of a hemolytic process with signs of active regeneration The number of red cells reticulocytes and hemoglobin concentration depend upon the severity of the disease and the ability of hematopoietic tissues to compensate for the destruction of red cells

The hemoglobin and red cells may be normal but usually are moderately to markedly reduced at birth The red cell count ranges from less than 2 million to as high as 5 5 million per cubic millimeter The most prominent feature in the blood smear is the large number of nucleated red cells in every stage of maturation This normoblastic outpouring reflects the accelerated regenerative activity of the bone marrow and extramedullary tissues The red cells are predominantly macrocytic with slight anisocytosis and poikilocytosis Spherocytosis is not a feature of the blood smear in Rh erythroblastosis as it is in erythroblastosis due to A B incompatibility

Although the number of normoblasts in the blood of the newborn infant does not exceed 10 per 100 white blood cells in the first two days of life in the severely affected infant it may rise to 5 or 10 nucleated red cells for every white blood cell In mild cases it is not increased above the normal Normoblastemia also may occur in infants with congenital heart disease in premature infants in infants whose mothers have diabetes in infants who have had fetal hemorrhage and in those with anoxia

Hemoglobin The hemoglobin varies from 5 gm per 100 ml to normal values of 15 to 18 gm Infants with marked anemia are critically ill with edema and those with congestive failure show a hemoglobin under 10 gm with a red cell count of 3 million or less Although the hemoglobin concentration is normally measured in blood pricked from the heel Mollison and Cutbush[30] have emphasized that it is obligatory to obtain the hemoglobin level from the cord blood Cord blood hemoglobin serves as a more accurate index of the status of fetal blood and a more precise guide to treatment than the higher values obtained from venous blood and even the more elevated reading acquired from blood obtained by finger puncture The reasons for these differences stem from the delayed typing of the umbilical cord blood the passage of placental blood into the fetal circulation and the rapid shift of plasma from blood vessels soon after birth Thus Mollison and Cutbush cite an instance of a cord blood hemoglobin value of 12 8 gm per cent with venous and capillary blood samples of 15 4 and 18 gm respectively Such discrepancies are frequently responsible for confusion in determining the need for an exchange transfusion within the first twenty four hours of life

The normal cord blood hemoglobin of 16 6 gm (16 55 $\pm$ 1 5) reported by Mollison and Cutbush[3] corresponds almost exactly with the values of 16 4 (16 4 $\pm$ 1 09) found in our laboratory The value of 13 6 gm per cent

patible matings about 50 per cent of the Rh positive fathers will be heterozygous so that half of the offspring may be Rh negative and hence without disease

Erythroblastosis due to the Rh factor in a population with about 15 per cent Rh negative persons occurs in 1 in 150 to 1 in 200 of all full term pregnancies The fact that only 1 in every 20 to 26 full term incompatible pregnancies re sults in an infant with erythroblastosis emphasizes the inability of most Rh negative mothers to produce Rh antibodies"[74] Even in Rh negative persons sensitized by transfusions with Rh positive blood there are many (about 10 per cent) who fail to develop antibodies even after a second transfusion or an in compatible pregnancy

Anti D (Rh_0) antibodies often in combination with anti C or anti E account for most of the cases of erythroblastosis due to the Rh factor Outside of the Rh factor and A and B factors which constitute the majority of cases c(hr′) E(rh″) Kell and a lesser number of blood factors outside of the Rh system mentioned previously (Chapter 6) account for the remaining 1 or 2 per cent of erythroblastosis

The frequency of erythroblastosis is directly proportional to the frequency of the Rh negative persons in any given population The incidence is less in Negroes in the United States with about 5 per cent Rh negative and is exceedingly rare in Chinese Japanese and American Indians among whom only 1 per cent or less are Rh negative"[75]

Prognostic Considerations and Family Patterns of Severity The titer of ma ternal anti Rh constitutes a primary factor in determining the prognosis in the fetus in utero If the anti Rh titer is less than 1 64 the chances are nearly 90 per cent that the baby will be in good condition at birth[4]

Certain predictions can also be made with respect to the severity of the disease in successive siblings The disease tends to be milder in the first affected infant than in those that follow However the trend of increasing severity is not uniform in every family and mild cases follow severe cases even if the infant with the severe case is stillborn About 5 to 10 per cent of first affected infants will be stillborn and of those born alive 40 per cent will not require treatment[44] If the first sibling is mildly affected then those that follow are likely to be mildly affected and the chances of a subsequent stillbirth are relatively low (about 2 per cent[11] If a previous baby was stillborn on an Rh basis there is about an 80 per cent chance that the next baby if spontaneously delivered will also be stillborn[11]

In another series it has been estimated that after a woman has had one stillbirth due to erythroblastosis the chances for the next Rh positive fetus are stillbirth 75 per cent born alive but with extreme anemia 15 per cent and born alive but with readily treatable disease 10 per cent When a woman has had two stillbirths due to erythroblastosis the chances for the next Rh positive fetus are stillbirth 90 per cent born alive but with extreme anemia 8 per cent, and born alive with readily treatable disease 2 per cent

Greater prognostic accuracy can be obtained by referring to severity of the disease in the immediately preceding sibling Thus there is a tendency for the very severely affected and stillborn infants to be followed by stillbirths and for

thrombocytopenia beyond the third or fourth day after an exchange transfusion with bleeding prompted a search for some other causative process Congenital thrombocytopenic purpura with a failure of megakaryocytes or a pyogenic infection should be considered in such instances [14]

Bilirubinemia Since kernicterus can be prevented in almost every case of erythroblastosis by keeping bilirubin levels below critical levels of 20 mg per 100 ml it is essential that bilirubin determinations be carried out from birth and frequently repeated during the first four or five days of life Micromethods are now available [1] which require only a small quantity of blood obtained from heel puncture Another technique has been described in which the plasma heme pigments measured spectroscopically also serve as a reliable guide to the need for one or more exchange transfusions [5] Bilirubin determinations however are more universally used

The cord blood bilirubin levels in most normal infants are under 3 mg per 100 ml The average is 2 2 mg (range 1 5 to 3 2) on the second day for the full term infant and approximately 11 mg (range 0 0 to 27) on the third to fourth day *in the premature infant Usually the bilirubin level begins to fall spontaneously* between the second and third day in the full term infant and on the fourth or fifth day in the premature baby The fall is more gradual in the premature infant and original cord values may not be reached until the tenth day or later

A knowledge of these normal values serves as a background for the fluctuations in the infant with erythroblastosis The cord blood bilirubin level of 3 5 and above in the affected infant is not much higher than that of the normal infant because of the clearing of this pigment in utero probably by the placenta Of greater importance in management is the rate of rise of bilirubin as determined by periodic estimations This ranges from 0 3 to 1 0 mg per hour depending upon the severity of the disease with the upper limit occurring in advanced disease In some instances the rise is gradual over the first two days and accelerates on the third or fourth day

REFERENCES

1 Aidin R Corner B and Tovey G Kernicterus and Prematurity Lancet 1 1153 1950
2 Allen F H Jr Early Jaundice in the Newborn Aids to Detection New England J Med 258 1302 1958
3 Allen F H Jr and Diamond L K Erythroblastosis Fetalis—Including Exchange Transfusion Technique Boston 1957 Little Brown & Co
4 Allison A C Danger of Vitamin K to Newborn Lancet 1 669 1955
5 Boggs T R and Abelson N M An Early and Reliable Guide in the Prognosis and Management of Erythroblastosis A M A Am J Dis Child 88 506 1954
6 Broman B Incompatibility of Blood Acta paediat 36 585 1948
7 Cathie I A B Breast Feeding in Erythroblastosis Fetalis Brit M J 2 650 1947
7a Chown B and Bowman W D The Place of Early Delivery in the Prevention of Foetal Death From Erythroblastosis Pediat Clin North America 5 279 1958
8 Coombs R R A Mourant A E and Race R R A New Test for the Detection of Weak and Incomplete Rh Agglutinins Brit J Exper Path 26 255 1945
9 Crigler J F and Najjar V A Congenital Familial Nonhemolytic Jaundice With Kernicterus Pediatrics 10 169 1952
10 Crosse V M Meyer T C and Gerrard J W Kernicterus and Prematurity Arch Dis Childhood 30 501 1955

therefore represents the lower level of normal for cord blood. These authors give values of 14.5 gm per cent for venous blood and slightly over 15.4 gm for capillary blood as the low limit of normal on the first day of life, which should serve as a guide to management when cord blood determinations are not available. Thus the range of normal hemoglobin values in newborn infants[30,31] may be stated as follows: *cord blood 13.6 to 19.6 gm per 100 ml; venous blood (first day)* 14.5 to 22.5 gm per 100 ml; blood obtained by skin prick (first day) 15.4 to 22.8 gm per 100 ml.

Severity of erythroblastosis has been classified on the basis of cord hemoglobin concentration: mild disease, 14 gm per 100 ml and above; moderately severe disease, 11 to 13.5 gm; and severe disease, less than 11 gm.

> In erythroblastosis the concentration of fetal hemoglobin is significantly lower than normal, whereas the concentration of adult hemoglobin is the same as in normal infants. There is no preferential destruction of fetal hemoglobin-containing erythrocytes in this disease; rather there is a preferential regeneration of adult hemoglobin in response to hemolysis. In infants born with normal hemoglobin levels the capacity to synthesize adult hemoglobin in amounts greater than normal appears to be an important mechanism serving to prevent anemia.[39]

Reticulocytes. The reticulocytes range above 6 per cent (upper limit of normal) in the infant with mild erythroblastosis and reach 40 to 50 per cent in those with severe disease. The reticulocyte count may also be increased when the cord blood hemoglobin is normal, indicating the ability of erythropoietic *tissue to compensate adequately for the demands of hemolysis.* A normal hemoglobin level, therefore, may be deceptive, and the need for repeated reticulocyte counts in such a case cannot be overemphasized as an index of underlying blood destruction. An infant with a normal hemoglobin level and an elevated reticulocyte percentage in the first two days of life may be in a precarious condition, for in many cases the hemoglobin is known to drop precipitously on the third day with the development of severe disease and the need for urgent treatment. The reticulocytes are of great value, therefore, in assessing the regenerative process since they are often found in increased numbers when nucleated red cells are within normal limits.[16]

Leukocytes. In infants with severe erythroblastosis the leukocytes vary from 15,000 to 30,000 per cubic millimeter. Higher counts have been recorded, but these include large numbers of nucleated red cells. Myelocytes are common. Erythrocytes which have undergone phagocytosis by monocytes or neutrophils are occasionally found in blood smears.

Platelets. The platelet count may be normal or diminished in infants with severe erythroblastosis. Thrombocytopenia may be sufficiently marked to cause petechiae and purpura. Usually the thrombocytopenia parallels the degree of the hemolytic process.[41]

PLATELET CHANGES FOLLOWING EXCHANGE TRANSFUSION. Desforges and O'Connell observed[13] low platelet levels several days after an exchange transfusion (as low as 50,000 per cubic millimeter and less in some cases), with a gradual rise beginning with the third day and restoration of normal values in about a week. Despite lowered values there was no bleeding tendency. The persistence of

34 Odell G B The Dissociation of Bilirubin From Albumin and Its Clinical Implications J Pediat 55 268 1959
35 Perlstein M Infantile Cerebral Palsy In Levine S Z and others (editors) Advances in Pediatrics vol 7 Chicago 1955 Year Book Publishers Inc p 209
36 Pickles M M Haemolytic Disease of the Newborn Springfield Ill 1949 Charles C Thomas Publisher
37 Potter E Diagnosis of Erythroblastosis Fetalis in the Macerated Fetus Arch Path 41 223 1946
38 Schulman I and Smith C H Fetal and Adult Hemoglobin in Hemolytic Disease of the Newborn A M A Am J Dis Child 87 167 1954
39 Silverman W A Andersen D H Blane W A and Crozier D N A Difference in Mortality Rate and Incidence of Kernicterus Among Premature Infants Alloted to Two Prophylactic Antibacterial Regimens Pediatrics 18 614 1956
40 Stamey C C and Diamond L K Congenital Hemolytic Anemia in the Newborn A M A J Dis Child 94 616 1957
41 Stefanini M and Dameshek W The Hemorrhagic Disorders New York 1955 Grune & Stratton Inc
42 Vaughan V C III Current Status of Hemolytic Disease of the Newborn Postgrad Med 18 115 1955
43 Vaughan V C III Allen F H Jr and Diamond L K Erythroblastosis Fetalis Further Observations on Kernicterus Pediatrics 6 706 1950
44 Walker W and Murray S Haemolytic Disease of Newborn as a Family Problem Brit M J 1 187 1956
44a Wheeler W E and Scholl M L L Determination of Anti Rh Antibody in Infants With Erythroblastosis Fetalis Am J Dis Child 74 274 1947
45 Wiener A S Nappi R and Gordon E B Studies in Rh Sensitization Methods Effect of Rh Negative Pregnancies on Rh Antibody Titer Blood 6 522 1951
46 Wiener A S Nappi R and Gordon E B Studies in Rh Sensitization Importance of the Titer of Rh Antibodies in the Sensitized Pregnant Rh Negative Woman for Prognosis Am J Obst & Gynec 63 6 1952
47 Gellis S S (editor) Year Book of Pediatrics 1957 1958 Series Chicago Year Book Publishers Inc
48 Zuelzer W W and Mudgett R T Kernicterus Etiology Study Based on an Analysis of 55 Cases Pediatrics 6 452 1950

11 Davies B S Gerrard J and Waterhouse J A H The Pattern of Haemolytic Disease of the Newborn Arch Dis Childhood 28 466 1953
12 Day R L Inhibition of Brain Respiration in Vitro by Bilirubin Reversal of Inhibition by Various Means A M A Am J Dis Child 88 504 1954
13 Desforges J F and O Connell L G Hematologic Observations of Erythroblastosis Fetalis Blood 10 802 1955
14 Diamond L K Clinical Pathological Conference J Pediat 50 490 1957
15 Forrester R M and Miller J The Dental Changes Associated With Kernicterus Arch Dis Childhood 30 224 1955
16 Gerrard J W Kernicterus Brain 75 526 1952
17 Gerver J M and Day R Intelligence Quotient of Children Who Have Recovered From Erythroblastosis Fetalis J Pediat 36 342 1950
18 Gunn C K Hereditary Acholuria Jaundice in the Rat Canad M A J 50 230 1944
18a Haberman S Krafft C J Luecke P E and Peach R O ABO Isoimmunization the Use of the Specific Coombs and Heat Elution Tests in the Detection of Hemolytic Disease J Pediat 56 471 1960
18b Harris R C Lucey J F and MacLean J R Kernicterus in Premature Infants Associated With Low Concentrations of Bilirubin in the Plasma Pediatrics 21 875 1958
19 Holman G H Studies on Physiologic Hyperbilirubinemia of Negro and White Premature Infants Pediatrics 22 1115 1958
20 Hsia D Y Y Allen F H Jr Gellis S S and Diamond L K Erythroblastosis Fetalis Studies of Serum Bilirubin in Relation to Kernicterus New England J Med 247 668 1952
21 Hsia D Y Y Hsia H H Gofstein R M Winter A and Gellis S S Determination of Concentration of Bilirubin in Serum Rapid Micro Method Employing Photoelectric Colorimeter Rapid Micro Method Employing Color Standards Pediatrics 18 433 1956
22 Johnson L Sarmiento F Blanc W A and Day R Kernicterus in Rats With Inherited Deficiency of Glucuronyl Transferase A M A J Dis Child 97 591 1959
23 Levine P The Mechanism of Isoimmunization by the Rh Factor of Red Blood Cells Arch Path 37 83 1944
24 Levine P Burnham L Katzin E M and Vogel P The Role of Isoimmunization in the Pathogenesis of Erythroblastosis Fetalis Am J Obst & Gynec 42 925 1941
25 Levine P Vogel P and Rosenfield R E Hemolytic Disease of the Newborn In Levine S Z and others (editors) Advances in Pediatrics vol 6 Chicago 1953 Year *Book Publisher Inc*
26 Lindsay S Hemolytic Disease of the Newborn Infant (Erythroblastosis Fetalis) a Study of the Pathologic Lesions of Twenty Cases J Pediat 37 582 1950
27 Lucia S P and Hunt M L A Study of the Occurrence of Normal Rh Negative Infants Born to Sensitized Rh Negative Women J Pediat 37 599 1950
27 McElfresh A E Kurkcuoglu M Vaughan V C III and Armbruster R *Elution of* Anti A and Anti B Antibody From Erythrocytes of Incompatible Newborn Infants J Pediat 56 39 1960
28 Meyer T C and Angus J The Effect of Large Doses of Synkavit in the Newborn Arch Dis Childhood 31 212 1956
29 Mollison P L *Blood* Transfusion in *Clinical* Medicine ed 2 *Springfield Ill 1956* Charles C Thomas Publisher
30 Mollison P L and Cutbush M Haemolytic Disease of the Newborn Criteria of Severity Brit M J 1 123 1949
31 Mollison P L and Cutbush M A Method of Measuring the Severity of a Series of Cases of Hemolytic Disease of the Newborn *Blood 6 777 1951*
32 Morton J A and Pickles M M Use of Trypsin in the Detection of Incomplete Anti Rh Antibodies Nature 159 779 1947
33 Odell G B Studies in Kernicterus I The Protein Binding of Bilirubin J Clin Invest 38 823 1959

Although bilirubin is removed simultaneously with the red cells of the infant by exchange of plasma it frequently rebounds to approximately two thirds of pretransfusion levels because of pigment sequestered in the extravascular reservoirs *It has been demonstrated that bilirubin accumulates in the plasma during* exchange transfusion even while it is being removed and must therefore enter the circulation from the tissues[9] The bilirubin withdrawn from tissue spaces is supplemented by that resulting from the breakdown of transfused blood especially if it has been stored for some time [8] Bilirubin is most effectively removed by a slow exchange and with large volumes of blood Anti Rh antibodies are also inadequately removed by an exchange transfusion because of their wide distribution in extravascular spaces

In infants with Rh erythroblastosis the immediate objective is to replace the vulnerable Rh positive cells which are the sources of bilirubin with the calculated amount of Rh negative cells The concurrent aim is the removal of pigment from the intravascular and extravascular fluid compartments This procedure is usually accomplished expeditiously within an hour or an hour and a half In the deeply jaundiced patient in whom correction of the anemia may not be so important as the removal of bilirubin an interval of about one half to one hour may be permitted to elapse between each volume of the exchange This delay will depend upon the condition of the infant and the general over all care in the treatment room

The blood volume also can be adjusted by exchange transfusion especially in infants with cardiorespiratory embarrassment Infants with cord blood hemoglobin levels of 8 gm per 100 ml or less are often distressed at birth and can be shown to have elevated venous pressure and often cardiac failure[33] One factor which may contribute to excessive blood volume and circulatory overload is the sudden passage of placental blood into the newborn infant at birth—hence the precaution of clamping the cord at the earliest moment In this type of case every effort is made to reduce blood volume and venous pressure by establishing a deficit before beginning the exchange transfusion The venous pressure is measured at intervals during the exchange and appropriate deficits are created as necessary Artifacts in venous pressure readings do occur If the pressure is unusually high the pulse and respiration will be increased Therefore these should be used together with venous pressure readings as criteria for the need to establish a deficit The reduction in blood volume and correction of the anemia by an exchange transfusion at birth may be life saving in the severely anemic infant

Indications The indications for exchange transfusion reside in the demonstration by clinical and laboratory means that the disease in the infant is and may be expected to be of sufficient severity to lead to the afore mentioned complications To a large extent the criteria for immediate exchange stem from clinical and laboratory evidence that a severe hemolytic process has been going on in utero Thus clinical evidence of severe disease such as pallor edema poor respirations and hepatosplenomegaly constitute indications for immediate exchange (jaundice at birth is practically never seen) Laboratory evidence of a severe prenatal hemolytic process includes the presence of anemia at birth (as

CHAPTER 10

Erythroblastosis Fetalis (Hemolytic Anemia of the Newborn Infant)—Treatment

Objectives of Treatment. The treatment of the infant with erythroblastosis fetalis has three major aims: (1) the prevention or control of cardiac failure (the major cause of death within the first twenty-four hours), (2) the prevention or control of bilirubinemia in an effort to prevent the occurrence of kernicterus (the major cause of death after forty-eight hours), and (3) the control of anemia (this is almost automatically associated with the first two aims). It will be seen that, except in the infant with severe anemia and cardiac failure, treatment is primarily oriented toward keeping the blood bilirubin low rather than toward achieving a high hemoglobin level.

To date the only effective therapy in controlling the afore-mentioned complications is exchange transfusion. In evaluating an infant for exchange transfusion, therefore, the physician must decide whether or not the disease state in the infant is severe enough to place him in danger of cardiac failure or subsequent hyperbilirubinemia of sufficient magnitude to cause kernicterus. Optimally, the decision concerning replacement transfusion should be made as soon as possible, since cardiac deaths often occur within the first few hours of life and since the prevention of bilirubinemia is much more efficient than the removal of the pigment once accumulation has been permitted to occur.

Treatment. The mainstay of treatment is the exchange transfusion, but its successful achievement depends upon many correlated items of procedure.

Exchange Transfusions

Rationale. Exchange transfusions accomplish the removal of all but a small fraction of the infant's own "destroyable" and sensitized Rh-positive red cells and their replacement by an adequate concentration of normal nonsensitized Rh-negative cells. Unless the infant's red cells are replaced, they will continue to be destroyed by the maternal anti-Rh antibodies, which persist in the circulation for four to eight weeks after birth. With a two-volume exchange (twice the blood volume of the infant), 85 to 90 per cent of the sensitized red cells are recovered.

(b) Hcp itosplcnoir eg ily
(c) Poor respiration
(d) J iundice
(e) Edema—with or without definitive signs of cardiac failure

2 Laboratory evidence of severe hemolytic disease
(a) Cord hemoglobin of 14 gm or below (less th in 14 5 gm venous blood or 15 gm capill iry blood)
(b) Reticulocytosis over 6 per cent
(c) Normoblastosis greater than 10 per 100 white blood cells
It should be remembered however that a low cord hemoglobin level may coexist with a low cord bilirubin level (below 3 mg) hence the greater importance of the former as a criterion for exch inge at birth

3 Factors favoring development of kernicterus
(a) History of severe disease stillbirth or kernicterus in a previous sibling
(b) Prematurity
(c) Maternal titer greater than 1 64

Indications for initial late exchange or repeated exchange

1 Continued signs of cardiac fa lure or severe illness
2 Indirect bilirubin levels ri ing at greater than 0 5 mg per cent per hour reaching 7 mg at six hours 10 mg at twelve hours and approaching 20 mg at any time

These indications may be restated as follows

An exchange transfusion is performed at birth in an infant with a positive Coombs test who also presents one of the following abnormalities
1 Clinical evidence of severe dise i e at birth with or without cardiac failure
2 Cord hemoglobin level of 14 gm and below
3 History of at least moderately severe disease of kernicterus or of stillbirth in a previous sibling
4 Prematurity e cept in the presence of the mildest disease
5 Cord bilirubin levels above 4 mg per 100 ml

A maternal titer of 1 64 or more should alert the pediatrician to the possible need for exchange transfusions However in institutions where facilities for sequential bilirubin determinations are limited exchange transfusions should be carried out at birth at this titer Wheeler and Ambuel have pointed out that 80 per cent of babies with a cord blood level above 4 mg per 100 ml eventually require an exchange transfusion whereas only 20 per cent of those with cord bilirubin below this level needed treatment These criteria for replacement transfusions are acceptable in most clinics for severe disease is usually antici pated if the infant is allowed to go untreated

If exchange transfusion is not performed at birth the bilirubin level is determined every four to six hours during the first day and every six to twelve hours on the second and third days Exchange transfusions are then given if

The co d hemoglobin level at which exch inge transfusions are recommended varies for the full term infant from below 11 gm per cent to below 15 5 gm per cent in different clinics The level of 14 gm per cent of hemoglobin i derived from the me in cord bl iod hemoglobin level obtained in a serie of 133 normal infant in our clin c (16 5 gm ± 1 5 gm)

determined by a low hemoglobin level in the cord blood), elevation of cord bilirubin (above 3 mg per cent), and manifestations of marked attempts at blood regeneration (normoblastosis, reticulocytosis).

The baby threatened by cardiac failure always has severe anemia. On the other hand, however, an infant with good powers of regeneration in utero may be born with a nearly normal hemoglobin concentration and look clinically well but may still be in severe danger of subsequent hyperbilirubinemia. This is due to the fact that the hemolytic process will proceed unabated postnatally but that the mechanism for excreting bilirubin will be inadequate. In utero, clearance of bilirubin is apparently carried out mainly by the placenta. Postnatally, however, bilirubin excretion is dependent upon the infant's liver function and the activation of a hepatic enzyme system which converts the insoluble indirect bilirubin to a soluble bilirubin diglucuronide (direct bilirubin) which may then be excreted. Development of efficient bilirubin excretion usually requires three to four days in a full term baby and longer in a premature infant. It is essential, therefore, to detect early those infants with a severe hemolytic process and to prevent the *occurrence of bilirubinemia by removing the Rh positive antibody coated cells* and replacing them with Rh negative cells.

In addition to the criteria described, there are other indications for immediate *exchange based upon the special susceptibility of certain infants to kernicterus.* These include premature infants, infants in a family with previous stillbirth and kernicterus, and infants born of mothers with very high antenatal antibody titers.

It has been well established that the incidence of kernicterus may be reduced to extremely low levels if the serum bilirubin is kept below 20 mg per cent. In the mature infant with erythroblastosis the likelihood of kernicterus has been estimated as about 1 in 10 for an indirect serum bilirubin of 20 to 25 mg per cent, about 1 in 5 for 25 to 30 mg per cent, and about 1 in 2 over 30 mg per cent.[11]

In the small premature or in any premature infant whose condition is poor, it may be necessary to postpone the exchange transfusion because of the risk involved. The known susceptibility of such infants to kernicterus aggravates the problem. Under these circumstances careful observation is necessary; successive *bilirubin determinations should be evaluated, and consultation with other physicians* may be necessary before treatment is undertaken.

Before treatment is contemplated, it is important to ascertain that the major portion of the bilirubin is of the indirect reacting type. Whereas this pigment is associated with brain damage, increased concentration of direct bilirubin indicates obstruction or an inflammatory process in the biliary system. An exchange transfusion for a high direct reacting bilirubin would be purposeless.

Indications for exchange transfusion may be summarized as follows:

Indications for immediate exchange at birth

Assuming that the *direct Coombs test* is positive, the indications for an immediate exchange are as follows:

1. Clinical signs
   (a) Pallor

juncture (that is in the older healthy infant with bilirubin levels fluctuating narrowly about 20 mg per 100 ml) the decision must ultimately be left to the clinical judgment of the attending physician who has full knowledge of both the hazard of hyperbilirubinemia and the potential risk of an exchange transfusion

PREMATURITY In a premature infant in whom the maternal titer is high that is reaching levels of 1 64 and in whom the cord blood bilirubin level is in the vicinity of 3 mg per 100 ml with hemoglobin levels of about 13 gm it may be necessary to postpone exchange transfusion if the infant s condition would be jeopardized by the procedure During the ensuing period successive bilirubin determinations are made and evaluated before treatment is undertaken This is not a simple matter and no unequivocal measurement can be employed to fill all contingencies The peculiar problems presented by the premature infant with erythroblastosis are similar to those in connection with exchange trans fusion in physiologic hyperbilirubinemia of full term and premature infants and are discussed further later in the chapter Parents should be informed that an exchange transfusion carries a small but definite mortality per se on which are superimposed the handicaps of prematurity Prematurity of two weeks or more constitutes a compelling indication for the prophylactic employment of exchange transfusions when the indications are otherwise equivocal [0]

INDUCTION OF LABOR In the infant with very severe disease the chances of sur vival diminish with the period of gestation It has been estimated that a woman who has had one stillbirth caused by Rh erythroblastosis has about 4 chances in 5 of losing her next Rh positive fetus if it remains in utero until term [3] Most intrauterine deaths occur before the thirty seventh week of gestation Induction of labor accordingly has been recommended at or after thirty seven weeks of gestation in sensitized Rh negative women when conditions are obstetrically favorable and the fetus is of good size and is viable Specifically the indications are history of a severely affected infant hydrops or stillborn baby a homozygous father or a rising maternal antibody titer or one that has already reached 1 64 Cesarean section is exceptionally advised if the mother s condition is unfavor able for induction of labor usually after a trial of pitocin and ruptured membranes Based on a repetitive history of stillborn infants and a rising ma ternal titer induction of labor has been recommended as early as thirty two weeks in selected cases [10] The present trend however is for early delivery for sensitized mothers in whom the prognosis for the infant seems good but at a time closer to term [50]

Since the baby may be premature and may have already sustained the effects of blood destruction exchange transfusion is prepared for in advance of delivery with a supply of type O Rh negative blood at hand compatible with the mother s serum The earlier fear of a high incidence of kernicterus in the slightly pre mature baby has now been dispelled by its known prevention with the use of mul tiple exchange transfusions

Whole blood versus sedimented red cells—the protective action of albumin Although whole blood has been almost universally used in replacement sedi mented blood has its advocates [3, 43] The advantage of sedimented red cells is

bilirubin rises to 7 mg at six hours and 10 mg or more in the first twelve hours even if the hemoglobin level is above 15 gm (peripheral blood). From one to four exchange transfusions may be required to keep the level of bilirubin below 20 mg per 100 ml.

It should be emphasized that after the initial cord blood hemoglobin level is determined subsequent hemoglobin levels serve no purpose in deciding on the need for exchange transfusions to prevent kernicterus. The later hemoglobin determinations merely indicate the need for correcting the anemia which can be accomplished by small transfusions of packed cells. The bilirubin level, however, remains the only available index of the need for an exchange transfusion.[50]

Specific problems. Specific problems relating to exchange transfusion include age at time of treatment, prematurity and induction of labor.

AGE AT TIME OF TREATMENT. The late development of kernicterus usually can be avoided by early vigorous treatment when the trend of bilirubin levels is definitely upward during the first twenty-four to forty-eight hours. Following this policy, the problem of an initial transfusion beyond the second day of life is usually never pressing. Boggs, for instance, has reported a cure in 53 of a group of 56 infants born to women who had had a previously affected infant provided the infant was first seen before the age of 12 hours.

Individual problems arise, however, after the first seventy-two to ninety-six hours, at which time the infant may be expected to begin excreting bilirubin momentarily. The question as to what age it is no longer beneficial to perform an exchange transfusion in the face of hyperbilirubinemia is most difficult to answer. No time limit in days can be set beyond which hyperbilirubinemia is no longer dangerous in the full term infant and in the premature infant it is even more difficult. According to one point of view,[57] exchange transfusions *need no longer be considered after the fifth day in a full term infant or after* the sixth day in a premature infant if hyperbilirubinemia has not yet developed. According to another view, no exchange transfusions are necessary in the full *term infant after the age of 5 days or in the premature infant after the age of 10 days.*[11]

On the other hand, the need for an exchange transfusion is not readily assessed if the bilirubin level, which has risen slowly in the first days, reaches levels somewhat below 20 mg per 100 ml, and if on the fourth day after birth the infant is vigorous, takes its feedings well, and shows no nervous system irritability. In such infants serial bilirubin levels must be observed through the first week of life if necessary. High bilirubin levels cannot be ignored at any specific number of days following birth. Exchange transfusions are indicated in the infant at 7 days of age, for example, if the bilirubin is in excessive concentration.[3] Patients have even been described in whom the early signs of kernicterus have been reversed by one or more exchange transfusions. Except for the established bilirubin level of 20 mg per 100 ml, there is no way of knowing at which of the lower levels a particular infant is in jeopardy of nervous system involvement. Infants with bilirubin levels greatly in excess of 20 mg have been known to be free of nervous system irritation, whereas some with much lower levels have had nervous system involvement. *Since there is no uniform* opinion at this

Sterile pack

Plastic catheters (polyvinyl)
2 infant feeding tubes sizes #K31 and #K3_
Sterile disposable plastic tubing (polyethylene) PE 190/S12 and PE 90/S12†
3 three-way stopcocks
15 cm stainless steel rule
2 metal basins
1 cut-down set
1 transfusion set
1_ syringes (10 ml.)
Rubber tubing (to fit the stopcocks)
Umbilical cord tape
Sterile drapes dressing to include 1 circumcision sheet and 2 gowns
Sterile jar 3 by 3 inches with Zephiran chloride solution
Sterile test tube with catgut with curved needle (plain 3-0)

Unsterile equipment

Operating board with facilities for heating hot water bottle aquamatic K pad
Blood warming apparatus§
Oxygen
Suction
Gravity pole
Waste bucket
2 Ace bandages (2 inch width)
1 circumcision board

Also available should be heparin solution 10 per cent calcium gluconate solution antibiotics and specimen tubes for coagulated and uncoagulated blood Blood for exchange transfusions should be as fresh as possible (no older than four days) to avoid the risk of dangerous increases of potassium in the plasma and should be properly warmed before administration The blood should never be placed in a water bath *above* 37 C because of the danger of causing hemolysis

The procedure for the exchange transfusion is as follows

1 On a fairly large sterile field all sterile equipment to be used should be laid out so that it will be within easy reach of the operator
2 The tubing should be rinsed inside and out with saline solution and smoothed
3 The three way stopcocks are then fastened in tandem and attached to a 20 ml syringe and to the catheter The discharge tube is attached to the proximal stopcock so that removal of the syringe or emptying of the tube will not allow air to fall into the distal stopcock. The transfusion recipient set is attached to the distal stopcock.
4 One of the basins should be filled with sterile saline solution The other should be filled with saline solution to which heparin solution has been added (1 300)
5 The syringes should all be rinsed with the heparin saline mixture
6 The baby should be completely immobilized on a Y board leaving the abdomen exposed The immobilization should allow for the provision of external heat suction and oxygen The abdominal wall should be prepared by cleansing and draping as for a laparotomy

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Pharmaseal Laboratories Glendale Calif
†Intramedic Clay Adams Inc New York, N Y
‡Gorman Rupp Industries Inc Belleville Ohio
§Hospital Instruments Co Scotia N Y

that the hemoglobin concentration is less likely to fall to levels requiring further transfusion. In this method approximately 100 to 150 ml of the supernatant ACD plasma layer is removed *from a bottle of previously stored Rh negative* blood.*

One of the primary objectives of exchange transfusions is the removal of maximal amounts of bilirubin already present in the plasma and that which enters the circulation from tissue depots during the exchange. Another indirect advantage is that plasma protein, especially albumin, which serves as a vehicle for binding bilirubin is supplied in larger quantity by whole blood than by the restricted amounts present in sedimented blood. Transfusion with whole blood receives renewed strong support from the demonstration of a protective effect of albumin against the development of kernicterus.[73, 75] The use of albumin solutions as an adjunct to exchange transfusions to bind bilirubin in extravascular *spaces appears to have merit.*[35a]

Whole blood and packed red cells have been used in combination in a single procedure, with the latter employed for about one third of the exchange transfusion.[13] The use of whole versus sedimented cells deserves further clinical and experimental evaluation because of increasing interest in the possible advantages of the latter. Sedimented red cells have already found a place in exchange transfusions in infants with profound anemia who are often in cardiac failure and show a high venous pressure.[5] In this case a one to one and a half volume exchange is usually advisable because of the precarious state of the infant. Except for this emergency, whole blood is the preferred medium in exchange transfusions, especially in infants with elevated or rising bilirubin levels.[6]

*For the present, whole blood is recommended in exchange transfusions, es*pecially in infants with elevated and rising bilirubin levels.

Estimation of the amount of blood to be used. The infant's blood volume should be estimated on the basis of 85 ml per kilogram of body weight. The total amount to be used equals twice the blood volume. The amount of blood actually exchanged by alternate withdrawal and introduction equals the total minus amount initially withdrawn. The remaining donor blood (equal to the amount withdrawn initially) may be carefully given as a terminal transfusion *only* if the venous pressure is normal.

Example—3 kg infant

Blood volume 255 ml
Total blood ordered 510 ml
Initial withdrawal 30 ml
Exchange 480 ml

Procedure. Whenever possible the exchange transfusion should be performed by catheterization of the umbilical vein. The operator must be prepared, however, to do a cut down if necessary.

The necessary equipment should be completely assembled before the baby *is exposed.*

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* From a mixture of 500 ml of blood and 120 ml of ACD (acid citrate dextrose) anticoagulant.

should be obtained for various determinations (Coombs test total protein hematocrit hemoglobin bilirubin etc)

15 The catheter should be withdrawn the cord carefully tied and a sterile dressing applied to the area (If a second exchange is contemplated a saline dressing should be used)

16 Following the exchange prophylactic antibiotics are given 100 000 units of penicillin and 30 to 40 mg per kilogram streptomycin intramuscularly in divided doses every twelve hours for four to seven days

If the clinical condition of the infant indicates that a second exchange will probably be required the catheter may be filled with sterile heparinized saline solution plugged off and left in the vein covered with a sterile dressing A catheter should not be left in place more than eight to twelve hours Any prolonged stay may lead to infection Actually it is rather easy to reinsert a catheter into the vein previously used

The rapid movement of bilirubin into the plasma during or immediately following an exchange transfusion the so called rebound phenomenon" has prompted an interruption of the procedure for approximately one hour between the first and second volumes of blood Provided the infant is maintained in good condition this modification has proved very effective in cases in which the removal of bilirubin is the prime objective It is hoped that this device will reduce the need for repeated exchanges

Saphenous vein method In the method described by Wiener and co workers[5] the infant is bled from the radial artery while blood is introduced into the internal saphenous vein Although this procedure constitutes an effective method *of exchange transfusion the intermittent substitution method utilizing the um*bilical vein without the need for skin incision is the one in common use If the umbilical vein is definitely unavailable the femoral or saphenous vein is used instead

Heparinized blood Heparin instead of citrate has been employed as an anticoagulant for exchange transfusions[4 37 48] but not as yet on a widespread scale Heparinized blood has many advantages the blood is necessarily warm and not refrigerated since it is used within a short period after being drawn it avoids citrate and potassium intoxication frequent calcium gluconate injections are unnecessary and the blood is more concentrated with higher hemoglobin levels since dilution by acid citrate dextrose mixtures is avoided The obvious disadvantages are that appropriate donors must be immediately available since heparinized blood keeps poorly and that there is the rare possibility of a coexisting bleeding tendency in the infant Heparin is used in a dosage of 15 mg per 500 ml of blood To avoid the danger of hemorrhage 15 mg of protamine sulfate solution is administered via the umbilical catheter at the end of the procedure[3] This step is not always carried out

Unexplained death Exchange transfusions are not without their dangers and unexpected fatalities occur It has been estimated that the frequency is about 1 per cent of replacements Cardiac arrest has been attributed to hyperpotassemia hypocalcemia citrate toxicity and acidosis In any one case it is al

7 When everything is ready and after all equipment has been checked the procedure should be started Aspiration of the infants stomach is a useful preliminary procedure

8 The umbilical cord should be cut squarely 1 cm from the skin margin The umbilical vein should be identified (it is the largest of the three vessels and at the 12 o clock position) Any visible blood clots should be removed with forceps One edge of the vein should be picked up with mosquito forceps The catheter should then be inserted into the lumen of the vein for a centimeter or so Suction should be applied through the syringe and the cannula withdrawn so as to remove any deeper blood clots After making certain that the catheter has been rinsed clear it should be gently inserted into the vein until blood is obtained If difficulty is encountered slight caudad traction should be made on the vein and the catheter inserted and withdrawn until the proper channel has been found (The introduction of a catheter more than 7 5 cm beyond skin surface may place the tip within the heart) When the catheter is in the right position umbilical cord tape should be placed around the cord and cannula to provide an airtight seal As previously stated artifacts of venous pressure readings must be kept in mind and will be recognized if pulse and respirations are normal

9 The system should then be rinsed through with saline solution the stopcocks disconnected from the catheter and a venous pressure reading should be obtained by measuring the height of the column in the catheter against the steel rule placed just above the umbilicus or at the xyphoid process (Normal venous pressure is 4 to 8 cm)

10 The proper amount of blood should be withdrawn before any blood is administered Specimens both coagulated and uncoagulated should be obtained for various determinations For full term infants with high hemoglobin values and without cardiac failure 20 to 40 ml of blood may be withdrawn before any blood is given This deficit improves the efficiency of the exchange

11 The infant should then be given 20 ml of the donor blood Thereafter the exchange is continued with the alternate withdrawal and introduction of equal amounts of blood For the average baby 10 ml increments are quite satisfactory

12 Throughout the procedure care must be taken to keep syringes and tubing well rinsed to prevent clotting If the infant struggles or cries it may be an indication of an increased venous pressure This should be checked before proceeding A written record of the exact amount of blood introduced and withdrawn should be kept

13 The infant should then be given 1 ml of 10 per cent calcium gluconate solution after each 100 ml of blood introduced This should be diluted in saline solution and given over a period of several minutes with careful auscultation of heart sounds and rate during the administration

14 When the required amount of blood has been exchanged a final sample

Anemia in the Previously Treated Infant One or more supplementary trans fusions with packed cells (10 ml per kilogram) are given before discharge from the hospital to raise the hemoglobin to 10 to 12 gm per 100 ml Since anti Rh antibody persists for at least four to eight weeks following birth weekly hemo globin determinations are necessary during this period Transfusions of packed cells are given in the clinic in the first two or three weeks following discharge from the hospital when the hemoglobin level falls below 8 gm per 100 ml

After this period transfusions are usually unnecessary particularly if a reti *culocytosis appears unless the 8 gm concentration of hemoglobin is associated* with illness Transfusions above this level may suppress innate hemoglobin and red cell synthesis which is usually in progress between the sixth and eighth weeks of life Hematinics are without value in the prevention or treatment of anemia at this time

Hyman and Sturgeon[4] have shown that during the convalescent period of erythroblastosis fetalis there is often an increase in total hemoglobin despite a fall in hemoglobin levels This discrepancy is explained on the basis of growth since a simple calculation of blood volume will reveal that the infant is indeed regenerating hemoglobin which is not reflected in the reduced peripheral hemoglobin concentration

In severe erythroblastosis however anemia may persist for several weeks following dis charge from the ho pital and is stated to exceed that which is expected from continuing hemolysis *after successful replacement ha been achieved or from an expanding blood volume* due to growth of the infant This form of anemia has been attributed to bone marrow hypo- plasia as a complication of severe hemolytic anemia in the newborn infant[18] from suppression of the inherent blood formation due to the known inhibitory effects of transfusion[46] or from antibody specifically directed against the red cell in the fetus and extending into the neo natal period[45]

Comprehensive bone marrow examinations however do not explain the continuing anemia following the acute hemolytic period on the basis of a decrease in the number of normoblasts in the bone marrow [24] On the contrary serial examinations indicate that the percentage of normoblasts in the bone marrow is increased in proportion to the anemia and that a true aregenerative state appears to be a rare occurrence The persistent anemia following the acute hemolytic stage of erythroblastosis probably represents a diminished re activity of the bone marrow which may be more severe than is normally present

Anemia in the Previously Untreated Infant It is important to watch those babies closely after discharge who have not had previous exchange transfusions because their anemia was mild (10 to 11 gm per 100 ml) during the hospital stay In an occasional case a precipitous drop in the hemoglobin occurs in the second and third weeks of life Death from severe and progressive anemia may result from excessive blood destruction coupled with inadequate compensatory blood formation Transfusions to maintain adequate hemoglobin levels are urgent in these infants

Rupture of the Spleen Splenic rupture is a rare complication of erythroblas tosis occurring regardless of therapy Many factors have been incriminated extramedullary hematopoiesis increased phagocytosis weakening of the sup portive structures and the tendency to hemorrhage[35,44]

Management of the Infant With Erythroblastosis When a sensitized mother enters the hospital for delivery of her baby the pediatric service and the blood bank should be alerted immediately by the obstetric service At the time of de

most impossible to single out the causative factor Cases of cardiac arrest during exchange transfusion have been reported which were successfully treated by thoracotomy and cardiac massage [18,36]

Miscellaneous Treatment The following are some of the auxiliary measures suggested for treatment of patients with erythroblastosis

Neutralization of Antibodies with Haptens Lipoid extracts of Rh positive red cells (haptens)[10] have been prepared with the hope of neutralizing anti Rh antibodies in the mother without at the same time stimulating fresh antibody production Injections of this material have proved ineffective since immunization once it has been established appears permanent

ACTH or Cortisone The usefulness of ACTH or cortisone in the treatment of autoimmune acquired hemolytic anemia prompted its trial in mothers during pregnancy and in infants after delivery The results in the mother have been equivocal with claims however that the stillbirth and neonatal death rates were significantly reduced [3]

The use of ACTH as a substitute for exchange transfusions has met with failure despite transient increases in hemoglobin levels On the other hand there is evidence that as an adjunct to exchange transfusion therapy it reduces the need for repeating exchange transfusions Klingberg and Jones recommended the use of ACTH in a dosage of 12 5 mg every twelve hours by intramuscular in jection for five to seven days until signs of excess hemolysis and bilirubinemia occur More carefully controlled investigation is needed before consideration can be given to its use

Glucuronic Acid The administration of glucuronic acid by oral and intravenous routes has been advocated as a means of converting the insoluble indirect or free bilirubin which accumulates in the blood of infants with erythroblastosis and is associated with kernicterus to the water soluble direct bilirubin which is excreted by the liver Although lowering of in direct bilirubin has been reported by one group [13] it has not been substantiated by others This approach bypasses the essential enzymatic nature of bilirubin glucuronide formation Uridine diphosphate glucuronic acid is the source of the glucuronic acid of conjugated bili rubin and is transferred to bilirubin by an active enzyme system Since free glucuronic acid does not enter into the sequence of reactions the biochemical basis of this therapy is doubtful Furthermore it has been shown by animal experiments that kernicterus may develop despite a depression of serum bilirubin when glucuronic acid is given [28] It has been suggested that this agent produce a redistribution of bilirubin from the blood to other body compartments with a potential increase in concentration in central nervous system tissue Claims for the efficacy of glucuronic acid were finally withdrawn when it was shown that the same effects on the concentration of bilirubin in the serum resulted from comparable amounts of glucose and saline solution given intravenously

Results of Treatment With the reservations imposed by the uneven character of the severity of cases over the years the drop in over all mortality and incidence of kernicterus has nevertheless been obvious At The New York Hospital the im provement is reflected in the drop in mortality from 35 8 per cent in the pre exchange period (1932 to 1941) to 8 9 per cent in the exchange transfusion period (1947 to 1955) The liberal use of exchange transfusions has resulted in a striking drop in the incidence of kernicterus from 25 per cent in 25 infants with erythroblastosis in 1946 to a total absence of this complication in 31 infants in 1955

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Results of Treatment. With the reservations imposed by the uneven character of the severity of cases over the years, the drop in over-all mortality and incidence of kernicterus has nevertheless been obvious. At The New York Hospital the improvement is reflected in the drop in mortality from 35.8 per cent in the pre-exchange period (1932 to 1941) to 8.9 per cent in the exchange transfusion period (1947 to 1955). The liberal use of exchange transfusions has resulted in a striking drop in the incidence of kernicterus from 25 per cent in 25 infants with erythroblastosis in 1946 to a total absence of this complication in 31 infants in 1955.

The volume of blood used in exchange transfusion is best referred to in terms of multiples of the infant s own blood volume Thus for a child with an estimated blood volume of 250 ml the commonly used 500 ml replacement transfusion is a two volume replacement Such an exchange provides in 87 per cent replacement of the blood but the exchange of the red blood cells is dependent upon the hematocrit of the donor blood and upon the infant s initial hematocrit The higher the donor hematocrit and the lower the infant s initial hematocrit the higher the percentage of Rh negative cells which will be present in the infant s circulation at the end of the transfusion

For an exchange of 500 ml of blood with the average hematocrit of 36 per cent the hematocrit of Rh negative cell at the end of the transfusion (and this will be the infant s ultimate hematocrit when all of his own Rh positive cell have been destroyed) will be 32 per cent The percentage of Rh negative cells will range from 82 per cent for an infant with an initial hematocrit of 50 per cent to 96 per cent for an infant with an initial hematocrit of 10 per cent The size of the transfusion can also be determined according to the nomogram of Veall and Mollison which provide about 90 per cent replacement with donor blood and a final total hematocrit of 50 per cent in the infant

Treatment of the Infant in Cardiac Failure In most cases the signs of congestive failure (pallor dyspnea restlessness grunting respirations cyanosis) will be found in severely anemic infants Blood is withdrawn cautiously initially the amounts depending upon the elevation of venous pressure and general condition of the infant In infants with congestive failure usually 30 to 60 ml are withdrawn however as much as one third of the infant s blood volume may be withdrawn Sedimented cells (150 ml of supernatant ACD plasma removed) are recommended for the anemic infant with heart failure Replacement is carried out slowly and less than the calculated amount is given—approximately one to one and a half volumes of blood Administration of digitalis has been found unnecessary Antibiotics should not be administered through the umbilical vein for fear of an adverse reaction through an effect directly on the cardiac musculature

Exchange Transfusion in Physiologic Hyperbilirubinemia of the Full Term and Premature Infant In exceptional cases the indirect reacting bilirubin level in the normal full term infant and especially in the premature infant reaches critical levels comparable to those in infants affected with Rh and ABO erythroblastosis Neonatal jaundice not caused by blood group incompatibility is considerably more common in premature than in mature infants Exposure to concentrations of bilirubin of 20 mg and above carries the same hazard of kernicterus especially in the premature infant It may be that the peculiar susceptibility of the premature infant to kernicterus is due to the greater permeability of bilirubin through the blood cerebrospinal barrier in the premature infant than in the full term infant[5,6] Jaundice can be controlled by exchange transfusions as it is in the infant with erythroblastosis The exchange however must be carried out more slowly and re exchange may be required since the objective is the removal of bilirubin from the circulation rather than the removal of vulnerable cells as in cases of erythroblastosis The transfused blood should be of the same group and Rh subtype as the infant

livery a member of the pediatric staff should be in attendance Observation should be made of the color of the vernix and of the amniotic fluid They may be stained yellow in erythroblastosis fetalis When the baby is delivered the cord should be clamped immediately This is done for several reasons There is no advantage to the baby in a transfusion of antibody coated red blood cells and circulating antibody A great many of the more severely affected infants have an elevated venous pressure and would do better with a smaller blood volume It is estimated that the child may receive up to 100 ml of blood from the placenta

It is of the greatest importance that the umbilical cord be cut so as to allow a segment at least three inches long between the umbilicus and the tie The cord stump should be wrapped in gauze soaked in saline solution and great care must be taken to keep this tissue from becoming dried out

The child should be examined for manifestations of hemolytic disease jaundice (rarely immediately at birth) pallor petechiae edema and hepatosplenomegaly At the same time several blood specimens should be carefully collected *from the placental portion of the cord*

A 5 to 10 ml specimen of oxalated umbilical venous blood is examined for hemoglobin ABO group Rh type and direct antiglobulin * In the event of a positive Coombs test a serum bilirubin determination and a complete blood count including reticulocytes and normoblasts are carried out When there is any suspicion of erythroblastosis and the Coombs test has been erroneously reported as negative complete blood tests should nevertheless be carried out until the Coombs test is verified this should be done without delay When cord blood is not available venous blood or blood obtained by pricking the heel can be used for the initial tests The peripheral blood may be more suitable for study of red and white cell morphology and reticulocytes

After the preliminary studies have been performed the subsequent course may be decided It must first be determined of course whether or not the child is erythroblastotic

Group specific ABO Rh negative blood is usually given When a severely affected infant is anticipated group O Rh negative blood should be available *before delivery In any case* the blood used should always be compatible with the mother s serum The prospective donor s red blood cells also should be cross matched with the serum of the infant s mother and the compatibility should be confirmed by the indirect Coombs technique The mother s serum not only contains much more anti Rh antibody than the infant s serum but may contain antibodies other than Rh (O) It is only by such tests that other incompatibilities between mother s and infant s blood can be completely eliminated Group O Rh negative blood should be selected with low anti A and anti B titers and A and B substances (Witebsky) should be added

*At The New York Hospital 5 ml of clotted blood is sent to the blood bank for routine typing and the Coombs test The remainder of the blood specimen is saved for cross matching From 5 to 10 ml of oxalated umbilical blood is set aside for use of the pediatrician who carries out the serum bilirubin determination and the complete blood count including reticulocytes

appear to have outnumbered those due to the Rh factor With few exceptions the cases are confined to A or B offspring of group O mothers with the A factor predominating

The restriction of ABO erythroblastosis to 5 per cent of incompatible matings is due to several mechanisms by which fetal erythrocytes are protected from the action of maternal antibodies The most important probably relate to the neutral ization or fixation of potentially harmful maternal antibody by specific A or B blood group substances located in fetal and placental tissues [4, 9]

Clinical Features In about 50 per cent of cases the first born is usually affected in contrast to Rh incompatibility in which the first born escapes unless the mother has had previous transfusions The occurrence of A B erythroblastosis is therefore unanticipated unless a previous pregnancy has established the possi bility of incompatiblity from these blood factors

An important distinguishing feature of A B erythroblastosis is the development of jaundice within the first twenty four hours of life—hence the designation "ic terus precox" [1] Therefore jaundice appearing before 24 hours of age almost invariably means erythroblastosis due to A or B factor This must not be con fused with physiologic jaundice which appears on the second or third day when jaundice due to A B incompatiblity is frequently at its peak In the severe type of A B disease the icterus increases in intensity from the first day and may be as pronounced as corresponding cases of Rh incompatiblity Anemia at birth is rare and if it is present it is usually mild Splenomegaly is slight to absent Stillbirth and hydrops are rare and the prognosis for succeeding pregnancies is excellent A tendency toward early recovery is characteristic Hyperbilirubinemia can occur and if untreated results in kernicterus as in cases of Rh incompatibility The occurrence in the first born has been attributed among other causes to di verse A and B antigen like substances contained in material used for immuniza tion procedures

Laboratory Findings—Blood The hemoglobin level and red blood cell count are usually either normal or slightly reduced In most infants hemoglobin levels usually range from 15 to 18 gm per 100 ml in the first days of life There is sel dom a substantial fall in the hemogloin level during the course of the disease Increased normoblasts are less common than in Rh disease The white blood cell count is moderately elevated It is of interest that infants with erythro blastosis due to anti A antibody belong to subgroup A_1 [60]

The reticulocyte count is an essential test of special significance in A B erythroblastosis As in patients with Rh incompatibility reticulocytosis indicates overactivity of the bone marrow to compensate for the destruction of red cells of a shortened life span A normal hemoglobin level and reticulocytosis (over 5 to 7 per cent) indicate excessive blood destruction and are usually accompanied by rising bilirubin levels

The most characteristic hematologic finding in A B erythroblastosis is the presence of microspherocytosis as revealed by the blood smear This feature is in contrast with the almost uniform macrocytosis present in Rh disease Spherocytosis is best observed in the first days of life As would be expected there is an associated increase in osmotic and mechanical fragility Differentiation

It is still too early to judge the efficacy of exchange transfusions in this group of infants because of limited experience The problem is particularly trying in the premature infant whose physical condition does not warrant exposure to a prolonged technical procedure Under these circumstances evaluation of serial bilirubin determinations provides the only means of controlling the situation It may be necessary to defer treatment even with a rising bilirubin in the case of a small premature infant whose physical condition is precarious In general one cannot as yet be too dogmatic as to the exact bilirubin level at which an exchange transfusion should be undertaken in these infants [34] Clinical judgment in the individual case is needed and in the group of premature and full term infants a rigid yardstick cannot as yet be applied until a great deal more information is obtained

It is of interest that although available evidence has emphasized the susceptibility of premature infants with hyperbilirubinemia to kernicterus Holman[22] pointed out the difficulties of assigning critical levels over which the nonimmunized premature infant requires treatment by exchange transfusion For instance in his series of fourteen such premature infants with bilirubin concentrations exceeding 18 mg per 100 ml there was no clinical evidence of kernicterus in the neonatal period One explanation for this inconsistency was the preponderance of Negro infants who are notably more mature than white infants of the same weight Undoubtedly other auxiliary factors play a role in determining the ultimate concentration of bilirubin or are actually involved in producing kernicterus Prolonged follow up is necessary to determine minor and major neurologic sequelae in this group of infants The complexity of this subject is reflected in the comments on Holman s paper [22] Crosse and colleagues emphasize that replacement should be performed promptly in premature infants if the serum level of indirect bilirubin is over 18 mg per 100 ml or at a lower level if the bilirubin is rising rapidly Meyer[8] cautions that there might even be justification for replacement transfusion with a rapidly rising bilirubin before levels of 18 and 20 mg are reached Newns and Norron would give transfusions to those infants with a serum bilirubin of 30 mg and below if they become lethargic irritable difficult to feed or if there is a rapid rise of serum bilirubin on the third or fourth day After reviewing their cases of hyperbilirubinemia without blood group incompatibility Mores and co workers[30] claim that prophylactic exchange transfusion in full term infants is useless in premature infants it is *theoretically unfounded and its pra tical value has not been proved not even by those per forming it* In general the trend seems to be to give exchange transfusions to affected infants with the reservation that critical levels cannot be stated categorically (For additional comments on physiologic hyperbilirubinemia see Chapter 8)

Exchange Transfusions as a Treatment of Poisonings The removal of toxic substances from the blood and tissues of children in acute poisoning has been achieved by exchange transfusion The procedure has been successfully used in children with a wide variety of poisonings including barbiturates boric acid [6] methyl salicylate poisoning [11] and ferrous sulfate poisoning [4]

ABO Erythroblastosis In recent years incompatibility within the ABO blood group system has been established as a common cause of erythroblastosis The failure to recognize ABO disease in the past was due to the fact that frequently it was so mild that it was commonly overlooked From the 20 to 25 per cent of heterospecific pregnancies in which fetal red cells possess an A or B factor not present in the mother s blood a substantial number of cases of erythroblastosis develop In approximately 95 per cent of these infants the blood type of the mother has been group O in the others A or B factor In recent years these

than in blood samples from infants 24 to 48 hours of age At The New York Hospital a record of the maternal major blood group is made available to the pediatrician during pregnancy Blood typing and the Coombs test are performed in cord specimens of all infants of group O mothers If a positive Coombs test is found a hemoglobin determination reticulocyte count and blood smear are carried out to look for nucleated red blood cells and spherocytes in an effort to establish the presence of active hemolytic disease in the infant

Maternal serum in cases of A B erythroblastosis contains natural saline active antibodies anti A and anti B which are normally present in group O individuals Only if the titer is excessively high (over 1 1024 against A agglutinogens and 1 512 against B agglutinogens) may it help in diagnosis[56]

Mothers of affected infants also possess hyperimmune or incomplete anti A and anti B antibodies which sensitize the red cells of the fetus The presence of immune antibodies in the blood of the mother with affected children can be demonstrated by their failure to be neutralized with soluble A and B group substances This is the basis of a test for the titration of anti A and anti B antibodies after partial neutralization of the naturally occurring antibodies[54] Maternal serum containing these immune antibodies is capable of hemolyzing the infant s cells in vitro[12] Added complement is sometimes necessary to demonstrate this property The immune bodies reach their peak during the first ten days postpartum and remain steady for the next four to six weeks After that the majority diminish slowly in titer[29]

Spontaneous agglutination of the infant s coated red cells termed conglutination can be demonstrated in A B erythroblastosis Conglutination refers to the tendency of thick suspensions of sensitized or coated red cells (with anti A anti B or Rh immune antibodies) to clump spontaneously in protein media such as adult serum plasma and bovine albumin and in colloidal media such as gum acacia[53]

One of the most reliable serologic methods for the diagnosis of A B erythroblastosis is the demonstration of free antibody in the infant s plasma capable of agglutinating adult erythrocytes of the same blood group as the patient[5] The presence of such homologous antibody is presumably derived from the transplacental passage of excess maternal antibody into the fetal circulation In addition to these features the red blood cells of the affected infant clump spontaneously in plasma or colloid media

Relation of ABO Compatibility and Rh Immunization It has been pointed out that erythroblastosis due to the Rh factor occurs more frequently when ABO compatibility exists between mother and child[30] In one series[31] ABO compatibility between the mother and child was found to be present in 80 per cent of an unselected obstetric population in contrast to 95 per cent in a group of sensitized Rh negative women who bore infants afflicted with hemolytic disease

Wiener[4] ascribed the protective action of a heterospecific pregnancy on the basis of competition of antigens Since both A and B are better antigens than Rh in man the antigenicity of the weaker antigen may be suppressed It seems more likely that the destruction of A and B cells leaves fewer red cells for Rh sensitization However exceptions have been reported in which erythroblastosis resulted from combined effects of Rh and hyperimmune anti A antibodies found in the mother[7] Neutralized group O Rh negative blood is the recommended treatment

from hereditary spherocytosis is sometimes difficult The absence of a positive Coombs test and the presence of the disease in other members of the family are important features in the diagnosis of hereditary spherocytosis

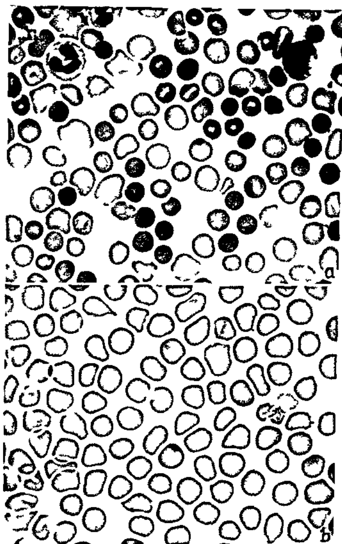


Fig 3 *A* Microspherocytosis in A B hemolytic disease *B* Uniform macrocytosis in Rh hemolytic disease (*From* Levine P Vogel J P and Rosenfield R E *Advances in Pediatrics* vol 6 Chicago 1953 Year Book Publishers Inc)

SEROLOGIC FINDINGS Without the use of refined methods the Coombs test with infant s cells (direct antiglobulin test) is generally negative or weakly positive in A or B incompatibility With specialized techniques however the Coombs test will be positive in as high as 90 per cent of cases of ABO erythroblastosis A strongly positive test is more likely to be observed in cord blood specimens

must be left to the clinical judgment of the attending physician who is aware both of the sequelae of nervous system damage and of the potential hazard of an exchange transfusion

In the anemic infant in whom the level of bilirubin is low or moderate and not rising small transfusions of packed group O cells of compatible Rh type are given in the first days of life

The blood used in all cases in the exchange transfusion should be fresh group O of the appropriate Rh type in which anti A and anti B antibodies have been neutralized with 10 ml of blood group specific substances A and B (Witebsky substances) If possible group O blood with low a and b" isoagglutinins is preferable As in cases of Rh sensitization re exchange may be necessary if hyperbilirubinemia persists exceeds original levels and shows evidence of rising to 20 mg per cent

As for subsequent pregnancies there is no fear of increasing severity as there is with Rh sensitization On the other hand since A B incompatibilities occur in at least 20 per cent of pregnancies it is a good plan to be alert to the recurrence of the disease However there is no evidence that antibody titers caused by heterospecific pregnancies ordinarily persist or affect unfavorably the outcome of future pregnancies [9a]

Differential Diagnosis The following conditions must be excluded physiologic jaundice hereditary spherocytosis congenital nonspherocytic hemolytic anemia neonatal hepatitis toxoplasmosis cytomegalic inclusion disease congenital syphilis and bacterial sepsis in the newborn infant (see Chapter 8) Jaundice should be controlled by exchange transfusion in patients with those conditions in which bilirubin of the indirect reacting type is increased before critical levels of hyperbilirubinemia appear and kernicterus threatens These include jaundiced infants with hereditary spherocytosis and congenital nonspherocytic hemolytic anemia The same consideration applies to newborn infants especially premature ones in whom physiologic jaundice is intense and whose blood shows excessive bilirubin concentrations [8] The differential diagnosis of jaundice in the neonatal period is given in greater detail in Chapter 8

REFERENCES

1 Adams J T Bigler J A and Green O C A Case of Methyl Salicylate Intoxication Treated by Exchange Transfusion J A M A 165 1563 1957

2 Allen F H M Jr Induction of Labor in the Management of Erythroblastosis Fetalis Quart Rev Pediat 12 1 1957

3 Allen F H Jr and Diamond L K Erythroblastosis Fetalis—Including Exchange Transfusion Technic Boston 1957 1958 Little Brown & Co

4 Amerman E E Brescia M A and Aftahi F Ferrous Sulfate Poisoning J Pediat 53 476 1958

4a Bentley H P Ziegler N R and Krivit W The Use of Heparinized Blood for Exchange Transfusion in Infants A M A J Dis Child 99 8 1960

5 Boggs T R Jr Clinical Experience With Hemolytic Disease of Newborn Infants J A M A 165 9 1957

6 Boggs T R Jr and Anrode H G Boric Acid Poisoning Treated by Exchange Transfusion Pediatrics 16 109 1955

7 Bowen W R Foter E and Waters W J The Protective Action of Albumin in Bili

Treatment It must be emphasized that the proper indication for exchange transfusion in infants with ABO erythroblastosis is the degree of bilirubinemia and not the anemia The prevention of kernicterus due to hyperbilirubinemia therefore constitutes the major objective in management of A B erythroblastosis as in Rh disease Exchange transfusion is performed and repeated if necessary to keep the level of indirect serum bilirubin below 20 mg per 100 ml Although the majority of infants with A B erythroblastosis are mildly affected and require no treatment they nevertheless bear scrupulous watching About 20 to 30 per cent of *infants with A and B incompatibility who develop icterus within the first* thirty six hours of life will need treatment This compares with an estimated 80 to 90 per cent of Rh positive infants who were born to sensitized mothers and who require one or more exchanges [40]

A level of 10 mg on the first day of life suggests the possibility of higher *levels in the next few days whereas the same level on the second or third day* indicates a more benign course [8] A reticulocytosis over 6 per cent and a rapidly rising bilirubin level of more than 0 5 mg per cent per hour constitute indications for treatment All infants with A B erythroblastosis should be closely observed during the first two days of life with measurement of daily hemoglobin and frequent serum bilirubin levels With increasing jaundice bilirubin levels may be required every six to eight hours If the bilirubin exceeds 10 mg per 100 ml in the first twelve to twenty four hours an exchange transfusion is required Deepening jaundice with a rise in bilirubin approaching levels of 20 mg on the second day of life also necessitates treatment

Because jaundice is frequently mild and is overlooked in the first days of life the pediatrician is often confronted with unexpected hyperbilirubinemia on the third or fourth day To avoid this contingency the ABO blood group and Rh typing of the mother and infant should be conspicuously placed on the chart and susceptible infants kept under scrutiny Any infant who is jaundiced before 24 hours of age who has an indirect serum bilirubin level exceeding 10 mg per 100 ml in the first twenty four hours and who is incompatible with the mother for A and B groups may be presumed to have erythroblastosis and should be treated

The management of situations in which the bilirubin level approximates 20 mg on the third and fourth days of life when jaundice has gone unnoticed for the *first forty eight to seventy two hours presents some difficulty In these infants the* rise in the concentration of bilirubin has probably been gradual There is no unanimity of opinion as to treatment in these circumstances Exchange transfusion in this group has not always been considered necessary because of the gradual rise in the concentration of serum bilirubin which eventually did not greatly exceed 20 mg per 100 ml [4] So many borderline cases of ABO erythroblastosis are encountered especially on the third and fourth day of life that it is difficult to abide by *hard and fast rules It is hoped that liver function will* be established at ninety six hours and that bilirubin levels will begin to drop Unfortunately this does not always occur Many regard it safe to postpone treatment for an additional day in a full term infant who is in good clinical condition and who is taking feedings well if the bilirubin slightly exceeds 20 mg on the fourth day As in cases of Rh erythroblastosis the ultimate decision in each case

31a Meyer T C A Study of Serum Bilirubin Level in Relation to Kernicterus and Prematurity Arch Dis Childhood 31 75 1956
32 Mollison P L Blood Transfusion in Clinical Medicine ed 2 Springfield Ill 1956 Charles C Thomas Publisher
33 Mollison P L and Cutbush M Haemolytic Disease of the Newborn Criteria of Severity Brit M J 1 123 1949
33a Mores A Fargasova I and Minarikova E The Relation of Hyperbilirubinemia in Newborns Without Isoimmunization to Kernicterus Acta paediat 48 590 1959
33b Nasralla M Gawronska E and Hsia D Y Studies on the Relation Between Serum and Spinal Fluid Bilirubin During Early Infancy J Clin Invest 37 1403 1958
34 Newns G H and Norron K R Hyperbilirubinemia in Prematurity Lancet 2 1138 1958
35 Odell G B Studies in Kernicterus I The Protein Binding of Bilirubin J Clin Invest 38 823 1959
35a Odell G B The Dissociation of Bilirubin From Albumin and Its Clinical Implications J Pediat 55 268 1959
36 Pew W L Cardiac Arrest During Exchange Transfusion J Pediat 47 645 1955
37 Pew W L Exchange Transfusion Using Heparinized Blood J Pediat 49 570 1956
38 Philipsborn H D Trusman H S and Greer D Jr Rupture of the Spleen a Complication of Erythroblastosis Fetalis New England J Med 252 159 1955
39 Robertson S E S A Study of the Anemia Following Exchange Transfusion in Haemolytic Disease of the Newborn M J Australia 43 250 1956
40 Schmid R Discussion on Observations on the Control of Bilirubinemia A M A J Dis Child 96 441 1958
41 Schmid R Jaundice and Bilirubin Metabolism A M A Arch Int Med 101 669 1958
41a Schwab M Perry R Boyer A Holt L E Jr Hallman N Backman A and Hjelt L The Influence of Sodium Glucuronate on Hyperbilirubinemia of the Newborn Further Observations Pediatrics 25 686 1960
42 Shumway C N Miller G and Young L E Hemolytic Disease of the Newborn Due to Anti A and Anti B Pediatrics 15 54 1955
43 Sisson T R C Whalen L E and Telek A A Comparison of the Effects of Whole Blood and Sedimented Erythrocytes in Exchange Transfusion Pediatrics 21 81 1958
44 Slotkowski E L and Hind A M Rupture of Spleen in Erythroblastotic Infant Pediatrics 4 296 1949
45 Smith C H Chronic Congenital Aregenerative Anemia (Pure Red Cell Anemia) Associated With Iso immunization by the Blood Group Factor A Blood 4 697 1949
46 Smith C H Schulman I Ando R E and Stern G Studies in Mediterranean (Cooley's) Anemia the Suppression of Hematopoiesis by Transfusions Blood 10 707 1955
47 Tovey G H A Study of the Protective Factors in Heterospecific Blood Group Pregnancy and Their Role in the Prevention of Haemolytic Disease of the Newborn J Path & Bact 57 295 1945
48 Valentine G H Heparinized Blood for Exchange Transfusions Lancet 2 21 1958
49 Vaughan V C Current Status of Hemolytic Disease of the Newborn Postgrad Med 18 115 1955
50 Vaughan V C III Management of Hemolytic Disease of the Newborn J Pediat 54 586 1959
50a Vaughan V C III Early Delivery for Hemolytic Disease of the Newborn? J Pediat 56 713 1960
51 Veall N and Mollison P L The Rate of Red Cell Exchange in Replacement Transfusions Lancet 2 792 1950
52 Wheeler W E and Ambuel J P The Efficient Use of Exchange Transfusions in the Treatment of Erythroblastosis Pediat Clin North America p 383 May 1957
53 Wiener A S Conglutination Test for Rh Sensitization J Lab & Clin Med 30 662 1945

rubin Toxicity in Newborn Puppies Program of the 29th Annual Meeting of the Society for Pediatric Research Buck Hill Falls May 8 9 1959 Abstract No 21 p 14
8 Brown A K and Zuelzer W W Studies in Hyperbilirubinemia Hyperbilirubinemia of the Newborn Unrelated to Isoimmunization A M A J Dis Child 93 263 1957
9 Brown A K Zuelzer W W and Robinson A R Studies in Hyperbilirubinemia Clearance of Bilirubin From Plasma and Extravascular Space in Newborn Infants During Exchange Transfusion A M A J Dis Child 93 274 1957
10 Carter B B Rh Haptens Its Preparation Assay and Nature J Immunol 61 79 1949
10a Chown B and Bowman W D The Place of Early Delivery in the Prevention of Foetal Death From Erythroblastosis Pediat Clin North America 5 279 1958
11 Chown B Prevention of Kernicterus as a Complication of Hemolytic Disease and Other Forms of Jaundice of the Newborn Blood 14 591 1959
12 Crawford A Cutbush M and Mollison P L Hemolytic Disease of the Newborn Due to Anti A Blood 8 620 1953
12a Crosse V M Walles G P and Walsh A M Replacement Transfusion as a Means of Preventing Kernicterus of Prematurity Arch Dis Childhood 33 403 1958
13 Danoff S Boyer A and Holt L E Jr The Treatment of Hyperbilirubinemia of the Newborn With Sodium Glucuronate Pediatrics 23 570 1959
14 Danoff S Grantz C Boyer A and Holt L E Jr The Conversion of Indirect to Direct Reacting Bilirubin in Vivo Science 127 759 1958
15 Day R L Discussion on Observations on the Control of Bilirubinemia A M A J Dis Child 96 440 1958
16 Diamond L K Discussion of Current Problems Regarding the Rh Factor Blood Spec Issue No 2 The Rh Factor p 180 1948
17 Diamond E F and DeYoung V R Acute Poisoning With Oil of Wintergreen Treated by Exchange Transfusion A M A J Dis Child 95 309 1958
17a Dillon H C and Krivit W Serial Study of Bone Marrow in Hemolytic Disease of the Newborn (Erythroblastosis Fetalis) Pediatrics 23 314 1959
18 Glassford G H and Motamedy F F Successful Treatment of Cardiac Arrest During Exchange Transfusion A M A J Dis Child 97 616 1959
19 Gunson H H An Evaluation of the Immunohematological Tests Used in the Diagnosis of A B Hemolytic Disease A M A J Dis Child 94 123 1957
20 Gunson H H Combined Rh and AB Hemolytic Disease of the Newborn Am J Clin Path 27 35 1957
21 Halbrecht I Role of Hemagglutinins Anti A and Anti B in Pathogenesis of Jaundice of the Newborn (Icterus Neonatorum Precox) Am J Dis Child 64 248 1944
22 Holman G H Studies on Physiologic Hyperbilirubinemia of Negro and White Premature Infants Pediatrics 22 1115 1958
23 Hunter O B Cortisone Therapy in Rh Incompatibilities J A M A 154 905 1954
24 Hyman C B and Sturgeon P Observations on the Convalescent Phase of Erythroblastosis Fetalis Pediatrics 16 15 1955
25 Jehu G Schmid R and Gellis S Administration of Glucuronic Acid to Icteric Newborn Infants Pediatrics 23 92 1959
26 Johnson L Sarmiento F Blanc W A and Day R Kernicterus in Rats With Inherited Deficiency of Glucuronyl Transferase A M A J Dis Child 97 591 1959
27 Klingberg W G and Jones B Corticotropin (ACTH) An Adjunct to Exchange Transfusion Therapy in Erythroblastosis Fetalis A M A J Dis Child 92 486 1956
28 Lathe G H Exchange Transfusion as a Means of Removing Bilirubin in Haemolytic Disease of the Newborn Brit M J 1 192 1955
29 Letters to the Editor Pediatrics 23 814 1959
30 Levine P Serological Factors as Possible Causes in Spontaneous Abortions J Hered 34 71 1943
31 Lucia S P and Hunt M L The Significance of ABO Compatibility and Its Relationship to the Intensity of Rh Immunization Blood 5 767 1950

CHAPTER 11

Anemias—General Considerations

Anemia may be defined as a condition in which the concentration of hemoglobin or the number of red blood cells either singly or in combination are reduced below normal The volume of packed red blood cells per 100 ml of blood measured by the hematocrit undergoes a simultaneous but not always parallel reduction The physiologic defect caused by the anemia is a decrease in the oxygen carrying capacity of the blood and a reduction in the oxygen available to the tissues Usually anemia is said to exist when the hemoglobin content of the blood falls below normal

Classification On an etiologic basis anemia results from an increased loss or destruction of red blood cells or a decreased rate of production Blood loss is due to acute or chronic hemorrhage and excessive hemolysis from intracorpuscular defects or extracorpuscular factors Impaired hemoglobin and red cell formation are due to a deficiency of substances required for their synthesis Defects of the red cells may be congenital or acquired but in either case their shortened life span frequently results in an anemia due to a failure of red cell production to keep pace with red cell destruction Erythropoiesis also may be depressed by toxic chemical or physical agents by space occupying or infiltrative lesions of the bone marrow or by unrecognized causes It will be noted that the classifications may overlap For example anemia may result from a combination of excessive destruction of erythrocytes with intracorpuscular defects as occurs in patients with hereditary spherocytosis and the hereditary hemoglobinopathies with inadequate compensatory erythropoiesis (For the hemolytic anemias see Chapter 15 for the hereditary hemoglobinopathies see Chapter 16)

Following is a classification of the anemias based on etiology

1 Blood loss—acute and chronic hemorrhage
2 Excessive blood destruction
   A Intracorpuscular or intrinsic defects usually hereditary
      (1) Hereditary spherocytosis thalassemia sickle cell anemia the hereditary hemoglobinopathies
      (2) Hereditary nonspherocytic anemia and elliptocytosis
      (3) Hemolytic anemia due to enzyme deficiencies

54 Wiener A S Competition of Antigens in Isoimmunization by Pregnancy Proc Soc Exper Biol Med 58 133 1945
55 Wiener A S Wexler I B and Brancato G J Treatment of Erythroblastosis by Exchange Transfusion J Pediat 45 546 1954
56 Witebsky E Interrelationship Between the Rh System and the A B System Blood Spec Issue No 2 p 66 1948
57 Gellis S S (editor) Year Book of Pediatrics 1956 1957 Series Chicago Year Book Publishers Inc
58 Zuelzer W W and Cohen F ABO Hemolytic Disease and Heterospecific Pregnancy Pediat Clin North America p 405 May 1957
58a Zuelzer W W and Kaplan E ABO Heterospecific Pregnancy and Hemolytic Disease I Patterns of Maternal A and B Isoantibodies in Unselected Pregnancies A M A Am J Dis Child 88 158 1954
59 Zuelzer W W and Kaplan E ABO Heterospecific Pregnancy and Hemolytic Disease Patterns of A and B Isoantibodies in the Cord Blood of Normal Infants A M A Am J Dis Child 88 179 1954
60 Zuelzer W W and Kaplan E *ABO Heterospecific Pregnancy and Hemolytic Disease* Pathologic Variants A M A Am J Dis Child 88 319 1954

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Anemias—General Considerations

Anemia may be defined as a condition in which the concentration of hemoglobin or the number of red blood cells either singly or in combination are reduced below normal The volume of packed red blood cells per 100 ml of blood measured by the hematocrit undergoes a simultaneous but not always parallel reduction The physiologic defect caused by the anemia is a decrease in the oxygen-carrying capacity of the blood and a reduction in the oxygen available to the tissues Usually anemia is said to exist when the hemoglobin content of the blood falls below normal

Classification On an etiologic basis anemia results from an increased loss or destruction of red blood cells or a decreased rate of production Blood loss is due to acute or chronic hemorrhage and excessive hemolysis from intracorpuscular defects or extracorpuscular factors Impaired hemoglobin and red cell formation are due to a deficiency of substances required for their synthesis Defects of the red cells may be congenital or acquired but in either case their shortened life span frequently results in an anemia due to a failure of red cell production to keep pace with red cell destruction Erythropoiesis also may be depressed by toxic chemical or physical agents by space occupying or infiltrative lesions of the bone marrow or by unrecognized causes It will be noted that the classifications may overlap For example anemia may result from a combination of excessive destruction of erythrocytes with intracorpuscular defects as occurs in patients with hereditary spherocytosis and the hereditary hemoglobinopathies with inadequate compensatory erythropoiesis (For the hemolytic anemias see Chapter 15 for the hereditary hemoglobinopathies see Chapter 16)

Following is a classification of the anemias based on etiology

1 Blood loss—acute and chronic hemorrhage
2 Excessive blood destruction
   A Intracorpuscular or intrinsic defects usually hereditary
      (1) Hereditary spherocytosis thalassemia sickle cell anemia the hereditary hemoglobinopathies
      (2) Hereditary nonspherocytic anemia and elliptocytosis
      (3) Hemolytic anemia due to enzyme deficiencies

B Extracorpuscular factors
- (1) Factors due to antibodies and isoagglutinins
- (2) Symptomatic hemolytic anemias
- (3) Lederer s anemia march hemoglobinuria paroxysmal cold hemoglobinuria
- (4) Miscellaneous causes such as infections and chemical and physical agents—hypersplenism

3 Decreased or impaired production
- A *Deficiency of substances required for hemoglobin and red cell formation* iron vitamin B folic acid ascorbic acid protein
- B Depression or inhibition of bone marrow
  - (1) Infection chemicals physical agents metabolic products
  - (2) Idiopathic depression and aplasia with or without congenital anomalies
- C Mechanical interference and replacement by abnormal cells leukemia Hodgkin s disease malignancies
- D Miscellaneous infection renal failure hypothyroidism

Orientation The anemias constitute the major segment of the blood disorders of infancy and childhood Anemia is a syndrome of multiple etiology whose origins are frequently difficult to discern for reasons inherent in the developmental processes of the pediatric period Many of the anemias undoubtedly are conditioned by factors operative in fetal life dating from the critical first months of gestation when the primary blood cell elements are established Others are influenced by the postnatal physiologic and anatomic changes which originate within and outside of the hematopoietic system Still others represent inherited inborn errors of metabolism such as the primaquine sensitive group of the hemolytic anemias The conditioning factors influencing the blood picture in the neonatal period and early infancy are listed below * Each of these items is discussed elsewhere in the text

1. The results of maternal isoimmunization by fetal blood factors
2. Bleeding from the placental surface abruptio placentae placenta previa and other complications of delivery
3. Fetal hemorrhage into maternal circulation
4. Anemia from congenital bleeding disorders overt or obscure hemorrhage
5. Fluctuations in blood volume as related to early or late tying of the cord
6. Substitution of adult for fetal hemoglobin
7. The aregenerative phase of erythropoiesis which characterizes the early months of life
8. *The possibility that a blood dyscrasia may represent a congenital or developmental* defect
9. The effects of prematurity
10. The effects of rapid body growth
11. Tendency for the hematopoietic system to react excessively to a stimulus reactivation of extramedullary foci of hematopoiesis
12. Inadequate fetal stores of iron from maternal deficiency of this mineral
13. Hemolytic anemia from maternal transmission of ingested drugs or chemical compounds

Diagnosis The problems of diagnosis in the infant and young child are complicated by the fact that the anemias tend to develop insidiously so that the

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* Based in part on Smith C H Anemias in Infancy and Childhood Diagnostic and Therapeutic Considerations Bull New York Acad Med 30 155 1954

complete hematologic picture with its specific criteria may be slow in emerging Despite these apparent difficulties it is possible with minimal laboratory equipment to arrive at a diagnosis by the judicious appraisal of data derived from a variety of sources such as the following *

1 History and physical examination
2 Basic blood studies complete blood count reticulocytes interpretation of blood smear
3 Comparison with normal range of blood values for each age period
4 Etiologic classification
   A Blood loss
   B Excessive blood destruction and hemolysis of extracorpuscular and intracorpuscular origin
   C Bone marrow depression or infiltration
   D Deficiency of building materials for hemoglobin or red cell formation
5 Reference to age periods of most frequent occurrence
6 Bone marrow examination
7 Roentgenographic examination
8 Study of hereditary factors
9 Therapeutic response to iron folic acid vitamin B_{12} liver transfusion
10 Specialized laboratory procedures
   A Essential
      (1) Stool guaiac test
      (2) Serum bilirubin
      (3) Blood urea nitrogen
      (4) Coombs test
      (5) Red cell fragility
      (6) Hemoglobin electrophoresis
   B Helpful
      (1) Gastric acidity
      (2) Stool urobilin
      (3) Serum iron and latent iron binding capacity
      (4) Bone marrow iron

The large number of items included in the list illustrates the wide scope of available information and the simpler technical procedures upon which the diagnosis is based However an insight into the nature of the anemia actually requires that relatively few of these topics be probed Under ordinary circumstances a thorough history physical examination and basic blood studies in conjunction with a simple classification based on etiology are usually sufficient to arrive at a correct diagnosis

History and Physical Examination Interrogation along broad lines of causation should include the following any change of behavior pattern such as irritability anorexia inactivity fatigability onset of pallor prematurity rate of weight gain quantity of milk ingested and attitude toward solid food Inquiry should also extend into a history of exacerbations of pallor and jaundice hematemesis loss of blood from the bowel infection with animal parasites allergy ingestion of drugs or household products known to depress hematopoiesis or to cause he

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molysis exposure to radiation frequency of respiratory infection skeletal pain joint swellings renal disease and purpura

Physical examination should include scrutiny of such diverse features as pallor jaundice skin pigmentation enlargement of lymph nodes and spleen petechiae and purpura In iron deficiency anemia acute leukemia and severe loss of blood the skin has a waxy whiteness Cardiac enlargement and signs of cardiac failure may be present A loud systolic murmur best heard at the apex and less commonly over the pulmonic area is frequently regarded as being of organic origin before the underlying severe anemia is discovered These murmurs are of hemic origin and usually disappear following transfusions In patients with advanced Cooley s anemia the facies have a characteristic appearance In this disease as well as in other conditions requiring multiple transfusions such as aplastic anemia and pure red cell anemia pallor is replaced by dark pigmentation and bronzing of the skin In patients in whom hemosiderosis is present and at times coexists with hemochromatosis sexual retardation is noted Increasing jaundice from the first day of life differentiates erythroblastosis fetalis from its slower development in icterus neonatorum Eye ground and nervous system changes are to be looked for in patients with sickle cell anemia Leg ulcers occur rarely in sickle cell anemia in children

Splenomegaly is most noticeable in infants with disorders attended by blood destruction such as erythroblastosis hereditary spherocytosis acquired hemolytic anemia and severe Cooley s anemia The spleen is occasionally enlarged in patients with iron deficiency anemia and those with megaloblastic anemia of infancy It is not palpable in infants with aplastic hypoplastic or pure red cell anemia It should also be remembered that a spleen palpable about 1 to 3 cm below the costal margin is present in a substantial number of otherwise normal infants and children The soft edge of the spleen in these children is in contrast with the hard edge found in patients with pathologic conditions Enlargement of the liver is occasionally found in patients with congestive heart failure and in those with sudden severe anemia from any cause It is often enlarged in patients with the hemolytic anemias especially in those receiving frequent transfusions and who have developed hemosiderosis and hemochromatosis

Except for the anemia associated with infection or with leukemia lymphadenopathy is not a conspicuous feature of anemia Enlarged cervical nodes frequently associated with a palpable spleen occur in the child with anemia due to repeated infections of the upper respiratory tract The superficial lymph nodes may be slightly enlarged in patients with severe Cooley s anemia and sickle cell anemia but not in those with the other intrinsic anemias A generalized lymphadenopathy in the presence of a refractory anemia and leukopenia should arouse suspicion of leukemia

Age Incidence in Relation to Diagnosis When infection which is responsible for the major number of anemias in the pediatric age group is excluded as an etiologic factor the remaining hematologic disorders can be grouped for orientation according to the age periods of their most frequent incidence newborn infancy and childhood

In the newborn infant the anemia is usually due to erythroblastosis fetalis If

this can be eliminated blood loss is to be considered Acute fetal blood loss during labor and delivery frequently resulting in posthemorrhagic shock may be due to fetal bleeding from the placenta occult transplacental loss of fetal blood into the maternal circulation or rupture of a normal or shortened umbilical cord[6]

Anemia due to iron deficiency occurs most commonly between 6 months and 2 years of age especially in the rapidly growing infant in the premature infant and in any infant whose dietary iron is restricted Iron deficiency anemia also occurs during infancy due to chronic gastrointestinal bleeding from embryonic structural defects such as diverticula Megaloblastic anemia of infancy occurs chiefly between the ages of 2 and 18 months Pure red cell (aregenerative) anemia is usually apparent at the age of 2 to 3 months or later in the first year When the infant is at approximately 6 months to 1 year of age the clinical and hematologic features of severe Cooley s anemia sickle cell anemia and hereditary spherocytosis are sufficiently conspicuous to be diagnosed They can be distinguished even earlier in suspected cases because of a familial background

Aplastic anemia hypoplastic anemia and Fanconi s anemia syndrome (aplastic anemia combined with multiple congenital anomalies) usually manifest their complete clinical and hematologic features after 3 years of age At about this time too Banti s disease and other disorders are encountered which are associated with splenomegaly and varying degrees of a hypersplenic blood picture such as that seen in Gaucher s disease It must be emphasized that the onset of the blood disorders first recognized in older infants and young children precedes by a variable period the time when the distinguishing features are fully established and unequivocal

Normal Blood Values in Infancy and Childhood Because of developmental changes anemias in infancy and childhood must necessarily be interpreted in the light of the contemporary hematologic framework within which they are encountered The range of normal values for the respective age periods which serve as a basis for evaluating an anemia are summarized in Table 5 A knowledge of the values for hemoglobin and red cells at birth and in the early newborn period are essential for an awareness of an anemia due to erythroblastosis or blood loss

For comparative purposes the average hemoglobin level on the first day of life may be regarded as 20 gm per 100 ml of blood with values ranging from 18 to 22 gm and the red cell count as 5 500 000 per cubic millimeter with a range from 5 to 6 million During the first two years of life the normal hemoglobin content ranges from approximately 10 to 12 5 gm per 100 ml of blood with an average value of 11 gm and between 5 and 10 years of age from 13 to 13 5 gm during puberty it rises gradually to the adult value of 14 5 gm per 100 ml A count of 4 million red cells per cubic millimeter of blood may be regarded as the lower limit of normal for the older infant and child In normal newborn infants the number of nucleated red cells in the first few days of life ranges from 3 to 10 per 100 white blood cells

Volume of Packed Red Cells (Hematocrit) In all anemias the measurement of the volume of packed red blood cells by the hematocrit constitutes an important and essential guide for diagnosis and therapy This determination reflects

the total mass of cells in a unit volume of blood and has proved to be of fundamental value in the study of all anemias in which considerable alterations occur

*Table 5 Normal Blood Values Significant in Diagnosis of Anemias in Infancy and Childhood**

Hemoglobin	
1st day	20 gm (18 to 22 gm)
2 wk	17 gm
1st and 2nd yr	11 gm (10 to 12 5 gm)
3 to 5 yr	12 5 to 13 gm
5 to 10 yr	13 to 13 5 gm
10 yr	14 5 gm
Red blood cells	
1st day	5 500 000 (5 to 6 million)
2nd wk	5 000 000
Older infant and child	4 000 000 per cubic millimeter lower limit of normal
Nucleated red cells	
Average—3 to 10 per 100 white cells (birth to 4 days of life)	
Reticulocytes	
0 5 to 1 5 per cent (6 per cent upper limit of normal—from birth to 4 days of life)	
(Below 0 5 per cent in aplastic and hypoplastic anemia increased in hemolytic anemias in deficiency anemia rise from low to high levels with treatment)	
Volume of packed red cells (hematocrit)	
Infants 1 mo to 2 yr	34 per cent
Children 2 yr to 12 yr	36 per cent
Older children	40 per cent
Serum bilirubin	
Newborn full term infants	2 to 8 mg per cent
Newborn premature infants	*1 to 15 mg per cent*
(Values given for both full term and premature newborn infants are the low values at birth rising to maximum during first week of life)	
Normal infants and children	Under 1 mg per cent
(Hemolytic anemias—elevated total bilirubin predominantly indirect fraction)	
Fragility test	
Normal range	0 425 to 0 325 per cent sodium chloride
(Increased fragility in hereditary spherocytosis and in some cases of acute hemolytic anemia decreased fragility in sickle cell anemia thalassemia [major and minor] and in iron deficiency anemia)	

* From Smith C H Anemias in Infancy and Childhood Diagnostic and Therapeutic Considerations Bull New York Acad Med 30 155 1954

in size shape and thickness of the red blood cells It has the advantage of being reliable and least subject to error in quantitative interpretation In patients with iron deficiency anemia for instance the extremely low hematocrit reflects the state of anemia more accurately than does the moderate reduction or even normal value in the number of red blood cells Also in the asymptomatic person with the trait of thalassemia the volume of packed red cells frequently remains at the same level in spite of wide fluctuations in the number of red cells

Because of the variations noted in available studies it is difficult to state the optimum packed cell volume for different age groups As a working basis a hematocrit of 34 per cent may be regarded as the lower limit of normal in infants after the first month of life 36 per cent from 2 to 12 years of age and 40 per cent in older children

Reticulocyte Count and Stain The reticulocyte count reflects the state of activity of the bone marrow hence this determination provides a useful guide in the diagnosis of both deficiency and hemolytic anemias and in gauging the response to treatment Normally from 0 5 to 1.5 per cent of the red blood cells are reticulated levels below 0 5 per cent represent inactive bone marrow A persistent depression in the percentage of the reticulocytes in spite of treatment occurs in patients with aplastic and hypoplastic anemia In those with anemia due to a deficiency a reticulocyte response from previously low levels follows appropriate treatment In patients with the hemolytic anemias the values for reticulocytes are constantly elevated and the high levels which are maintained prior to and without treatment represent intensified bone marrow regeneration In such cases the Coombs test will be positive in infants with erythroblastosis fetalis and acquired hemolytic anemia A negative test prompts a search for the diagnosis among the hereditary hemoglobinopathies primaquine sensitive hemolytic anemias nonspherocytic anemia and hereditary spherocytosis

Reticulocyte stain A glass slide is cleaned and polished Two or three drops of a saturated alcohol solution of brilliant cresyl blue are put near one end of the slide and allowed to dry Heavy streaks of precipitated stain are removed by rubbing with lens paper

A small drop of blood is placed in the center of the stained area and mixed with the stain by stirring with the edge of a second slide When thorough mixing has occurred, the stained blood is smeared across the remainder of the slide It is allowed to dry and then is counterstained with Wright s stain The number of reticulocytes per 1 000 red blood cells should be enumerated to obtain an accurate count. The result is expressed as a percentage

Relationship Between the Red Cell Count Hemoglobin and Volume of Packed Red Cells in Absolute Measurements Morphologically the anemias may be classified according to the size of the red cell normocytic microcytic and macrocytic A hemoglobin concentration within normal limits is called normochromic below normal hypochromic Hyperchromic anemias are nonexistent The increase in hemoglobin in the macrocyte may run parallel with the size of the macrocyte but does not exceed it Since the macrocyte is thicker than the normocyte the central pallor is less marked in the microcyte which appears dark when stained [1]

The indices calculated from the red cell count hemoglobin concentration and volume of packed red cells offer important clinical clues to treatment

Color index. The color index, a widely used measurement, is obtained by dividing the hemoglobin value in grams per 100 ml by the red cell count, both values being designated as a percentage of normal. It defines the hemoglobin content of the individual cells as compared with the content of a normal cell. A color index of approximately 1 either is normal or connotes a normocytic normochromic anemia; an index of below 0.8 characterizes a hypochromic anemia; an index above 1 denotes macrocytosis, which is usually associated with hypochromia.

Although the color index is, *in general, a valid expression of the relationship* between hemoglobin saturation and cell size, exceptions occur. In infancy and early childhood especially, the color index may be unreliable because of the normal fluctuations in hemoglobin and red cell counts which accompany growth.

$$\text{Color index} = \frac{\text{Hemoglobin (\% of normal [14.5 gm per 100 ml} = 100\%])}{\text{Red cells \% (5 million per cubic millimeter} = 100\%)}$$

More precise and instructive information regarding cell size and hemoglobin concentration in the younger age groups is obtained by measuring the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC).

Price-Jones curve. The Price-Jones curve is a graphic method of recording the diameters of red blood cells of various sizes from the stained blood smear.[7] In the majority of normal subjects, the peak diameters are approximately 6.69 to 7.7 *microns, which are taken as the upper and lower limits of normal.*[10] The average mean diameter is given as 7.2 microns. The sizes of the red cells are noted on the abscissa and the number of the cells (frequency) in each size group on the ordinate. The results are plotted in frequency curves. Figures or curves above or below the two peaks indicate macrocytosis and microcytosis, respectively. Anisocytosis is indicated by spreading of the base of the curve and flattening of the peak. The effects of treatment on macrocytosis in patients with pernicious anemia are particularly well demonstrated in the Price-Jones curve. Since measurement of the red cell diameters is a laborious procedure, the simpler measurement of the mean corpuscular volume is frequently substituted for it.

Red cell indices. The designation of the anemia according to size of the red cell and hemoglobin content serves to suggest specific blood disorders and their therapy.

MEAN CORPUSCULAR VOLUME (MCV). This represents the mean or average volume of a single red cell. *The result is expressed in cubic microns.*

$$\frac{\text{Hematocrit, per cent} \times 10}{\text{Red blood cell count, millions per cubic millimeter}}$$

Example:

Red blood cell count	5 million
Hematocrit	45%
MCV	$\frac{45 \times 10}{5} = 90$ cubic microns

Normal range MCV = 80 to 94 cubic microns

A MCV of more than 94 cubic microns indicate macrocytes 80 to 94 cubic microns normocytes less than 80 cubic microns microcytes

MEAN CORPUSCULAR HEMOGLOBIN (MCH) Mean corpuscular hemoglobin represents the average quantity (weight) of hemoglobin per individual red cell Results are expressed in micromicrograms

$$\frac{\text{Hemoglobin grams per 100 ml} \times 10}{\text{Red blood cell count millions per cubic millimeter}}$$

Normal range MCH = 27 to 32 micromicrograms

A MCH with normal hemoglobin content is called normochromic above normal hyperchromic below normal hypochromic

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC) This represents the average concentration of hemoglobin in the individual red cell as calculated from the amount of hemoglobin per 100 ml of cells rather than of whole blood The result is expressed as percentage

$$\frac{\text{Hemoglobin grams per 100 ml} \times 100}{\text{Hematocrit per cent}}$$

Normal range = 32 to 38 per cent

Red cells with MCHC of less than 32 per cent are hypochromic At approximately 34 per cent hemoglobin the normal erythrocyte contains a maximal number of hemoglobin molecules Hyperchromia in reality describes an increased intensity of staining of the red cells A cell with increased thickness as is observed in patients with hereditary spherocytosis gives the impression of hyperchromia (thickness above normal value of 2 millimicrons) MCH and MCHC measure the weight and concentration of hemoglobin respectively in the average red cell

The absolute values of the mean corpuscular hemoglobin concentration and the mean cell volume offer practical guides for diagnosis and therapy The combination of microcytosis (MCV below 60 cubic microns) and hypochromia (MCHC of less than 32 per cent) is indicative of iron deficiency Characteristic is a blood smear with a predominance of hypochromic microcytes and pronounced central pallor Thalassemia minor which possesses similar red cell indices and red cell morphology as iron deficiency anemia is characterized by a familial and hereditary pattern and the presence of A hemoglobin On the basis of the blood smear the two conditions are virtually inseparable In patients with the less common macrocytic anemias with mean red cell volumes ranging above 94 cubic microns restoration to normal values can be expected with liver vitamin B_1 and folic acid therapy Macrocytes may be fully saturated with hemoglobin or the pigment may be diminished in the latter case iron therapy is also indicated

In patients with chronic infections and in those with systemic diseases such as nephritis the red cells may be microcytic or normocytic with little or no decrease in the content of hemoglobin Patients with these infections and diseases fail to respond permanently to antianemia therapy unless the primary cause is treated Transfusions are symptomatically required for patients with these conditions as well as for those with the normochromic and normocytic anemias re

sulting from bone marrow depression These include aplastic and hypoplastic anemias and leukemia in which marrow cell displacement occurs A normocytic normochromic anemia in a child for which no definite hematologic basis can be discovered necessitates comprehensive survey for an underlying pathologic condition

Bone Marrow Examination Aspiration of the bone marrow constitutes a useful laboratory aid in the diagnosis of blood disorders The accessibility of the marrow its responses to stimuli producing depression or hyperplasia its availability for repeated examinations and the comparative ease of identifying the cellular elements account for frequency with which aspiration is performed[8] Disturbances of each of the principal blood elements are frequently reflected earlier or are more conspicuous in the bone marrow than in the peripheral blood In patients with leukemia for instance the bone marrow may be extensively infiltrated with leukoblastic cells whereas they appear in the peripheral blood in such scant numbers as to be overlooked In patients with hypoplastic aplastic anemias and in those with the hemolytic anemias bone marrow studies permit quantitative estimation of the cells types involved Bone marrow examination serves as a guide to therapy with antianemia agents and especially with the chemotherapeutic compounds in leukemia

NORMAL VALUES The figures in Table 6 represent the approximate range and average values of the cellular elements which one may expect to find in samples of the bone marrow obtained from normal infants and children Certain modifications must be made in view of the changing pattern of the percentages of the component cells The granulocytic series predominate in the first week or two of life By one month the lymphocytes increase in number and often extend beyond the 25 per cent level noted in Table 6 to reach levels of 50 per cent during the first year The presence of a lymphocytosis of this magnitude need not give rise to unnecessary concern provided the cells are morphologically normal Between 4 and 8 years of age the granulocytes equal the lymphocytes numerically and eventually predominate reaching adult values at about 12 years of age

Table 6 Normal Values of Cellular Elements in Bone Marrow in Older Infants and Children

	Range (Per Cent)	*Average (Per Cent)*
Myeloblasts	1 to 5	2
Myelocytes (including promyelocytes)	10 to 25	20
Nonsegmented polymorphonuclear cells (including metamyelocytes)	15 to 30	20
Segmented polymorphonuclear cells	5 to 30	25
Lymphocytes	5 to 25	13
Nucleated red cell (principally normoblasts)	15 to 30	20
Megakaryocytes	10 to 35 per cu mm	
Total nucleated cell count	100 000 to 200 000 per cu mm	

DIAGNOSTIC FEATURES IN THE BLOOD DISORDERS The individual cellular constituents in the bone marrow are in such close proximity to each other that it is difficult for one element to be affected independently of the others This total stimulation is best observed in patients with the hemolytic anemias and most regularly in those with acute hemolytic anemia In the latter regeneration of the red blood cells is associated with an increase in the number of granulocytes and platelets The reverse occurs in patients with aplastic anemia in whom values for the three types of blood cells are depressed simultaneously However other factors must operate in addition to anatomic proximity since involvement of a single type of cell may occur This is illustrated in persons with pure red cell anemia in whom the production of red blood cells is inhibited without equal depression of the other cellular elements Similarly selective hyperplasia occurs in persons with iron-deficiency anemia and in those with chronic loss of blood in whom the normoblasts and their immediate precursors alone are primarily increased

In patients with the hemolytic anemias (namely erythroblastosis fetalis hereditary spherocytosis acute acquired hemolytic anemia sickle cell anemia and thalassemia major) the bone marrow is hyperplastic and there is an increased

Table 7 Diagnostic Features of the Bone Marrow in the Common Anemias of Infancy and Childhood

Type of Anemia	*Diagnostic Features*
Hemolytic group (erythroblastosis fetalis hereditary spherocytosis sickle cell anemia thalassemia major acquired hemolytic anemia)	Hyperplastic marrow with increase of nucleated red cells chiefly normoblasts granulopoiesis may also be active occasionally during crises marrow aplastic instead of hyperplastic
Iron deficiency anemia	Hyperplastic marrow with increase in normoblasts granulopoiesis unchanged
Hemorrhagic anemia	Hyperplastic marrow with increase in nucleated red cells chiefly normoblasts granulopoiesis active in acute hemorrhage and unchanged in slow and chronic loss of blood
Megaloblastic anemia of infancy	Pattern similar to pernicious anemia in relapse megaloblastic type of erythropoiesis and changes in granulocytes resembling pernicious anemia
Aplastic anemia	Usually decreased cellularity to acellular sharp reduction in myeloid elements megakaryocytes and normoblasts increase in lymphocytes
Hypoplastic anemia	Hypoplastic marrow with pronounced depression affecting normoblasts mainly
Aregenerative (pure red cell) anemia	Normal marrow except for absence of nucleated red cells

From Smith C H Diagnosis of Anemias in Infancy and Childhood J A M A 134 992 1947

proliferation of normoblasts and to a lesser extent of erythroblasts Granulopoiesis may be active especially in persons with acquired hemolytic anemia In patients with iron deficiency anemia and in those with chronic hemorrhagic anemia the bone marrow is similarly hyperplastic and values for normoblasts are greatly elevated but granulopoiesis is normal In patients with acute hemorrhage however, the activity of the myeloid elements is also increased The increase of the nucleated red cells in patients with these disease processes in excess of 50 per cent of all the nucleated elements in the marrow constitutes an important diagnostic feature (Table 7)

Aplastic anemia is associated with profound anemia leukopenia neutropenia and thrombocytopenia The bone marrow shows a progressive decrease in cellularity and there is a sharp reduction in the myeloid elements megakaryocytes and nucleated red cells with a relative increase in normal lymphocytes Occasionally acute lymphoblastic leukemia in the leukopenic stage may simulate aplastic anemia and diagnosis is difficult without histologic examination of the bone marrow Bone marrow aspiration in patients with leukemia will reveal a cellular bone marrow in which the normal nucleated elements are replaced by primitive cells of the lymphoid or myeloid series With the progressive reduction in the number of platelets hemorrhages occur in patients with aplastic anemia which may simulate thrombocytopenic purpura The bone marrow in persons with idiopathic thrombocytopenic purpura reveals a hyperplasia of immature megakaryocytes In secondary thrombocytopenic purpura occurring in patients with leukemia and aplastic anemia megakaryocytes are reduced in number or are entirely absent in the counting chamber and the bone marrow smears

INVASION OF THE BONE MARROW BY FOREIGN CELLS Abnormal elements characterizing a specific pathologic condition occasionally appear in the bone marrow In younger age groups these consist most commonly of Gaucher and Niemann Pick cells the abnormal histiocytes of Letterer Siwe disease (nonlipid reticuloendotheliosis) and metastatic neoplastic cells such as are found in patients with neuroblastoma and lymphosarcoma

Characteristic tumor cells are found with such great frequency in patients with metastatic neuroblastoma that bone marrow examination is essential when this disease is suspected In one series of cases the abnormal cells were found in fifteen of thirty one infants and children Their presence is not dependent upon the existence of radiovisible bone lesions In patients with this condition ball like masses or clusters of large immature cells form syncytial masses or pseudorosettes with a mosaic pattern [5] The individual cells are large with nuclei possessing an immature chromatin pattern which stain deep blue The cytoplasm is scant faintly basophilic and without granules [3]

TECHNIQUE OF BONE MARROW ASPIRATION The sternal manubrium is rarely the optimal site for bone marrow aspiration in children Other areas offer the advantages of being less dangerous less painful and less emotionally traumatic to the patient Such sites are the lower thoracic or lumbar vertebral spinous processes anterior iliac crest posterior iliac crest and tibia In children older than 18 months to 2 years the spinous processes are most useful In younger children or infants selection of the iliac crest may expedite the procedure It is probably wise

*to reserve use of the tibia for exceptional circumstances since fractures have re*sulted from such punctures at this site

Skin preparation should include the use of pHisoHex as well as benzalkonium Anesthesia is obtained by the use of 1 per cent procaine solution Following *the intradermal injection of approximately 0 2 ml of the procaine solution* a short time interval is necessary to allow adequate anesthetic effect to occur If this step is observed it is possible to ensure an almost painless procedure The



Fig 4 Bone marrow smears in neuroblastoma *A* Note typical pseudorosette formation around a fibrillar network *B* Same formation as shown in *A* showing finer detail of individual cells in circular grouping (× 1200) (*A* Courtesy Dr Ralph L Engle Jr New York N Y)

subcutaneous tissue is next infiltrated with proper care being taken to avoid touching the periosteum Another short interval of time will allow further specific injection of the periosteal layer without discomfort to the patient

An 18 gauge 3/4 or 1 inch needle is used in the majority of children although a smaller gauge may be employed for very young infants Once the tip of the needle has been inserted through the periosteum the needle is inserted with added pressure until it is firmly fixed within the bone A sense of "give" is only occasionally felt as the needle enters the marrow cavity The point of insertion should be in the midpoint of the spinous process or just 1 cm below the lip of the ileum The angle of insertion should be perpendicular to the spinous process or to the flat surface of the ileum At all times the needle should be guarded at the skin s surface by the fingers of the operator s left hand to prevent it from slipping off the bony prominence while pressure is being exerted

The stilette is then removed and a 10 ml syringe is attached to the needle The actual aspiration of marrow contents is usually painful It may be wise at this point to warn the patient that if pain occurs it will probably signify the end of the procedure To minimize admixture with blood only 0 1 to 0 2 ml of marrow is withdrawn into the syringe *Release the suction in the syringe* This maneuver is necessary to avoid spreading the aspirate along the walls of the syringe when the needle is being removed from the bone Remove the needle still attached to the syringe from the puncture site and expel the aspirate onto a glass slide At this time speed is essential to avoid early coagulation and loss of cells especially megakaryocytes

A total nucleated cell count is made by diluting the fluid marrow in 3 per cent acetic acid as for a peripheral white cell count Cell types which may be particularly notable in the counting chamber include megakaryocytes and the large cells of Gaucher s and Niemann Pick diseases

Smears are made on glass slides which have been washed in 85 per cent alcohol Wright s stain is satisfactory for routine use

Contraindications to bone marrow aspiration are hemophilia and related coagulation disorders However the bone marrow can be safely aspirated in thrombocytopenic purpura Bone marrow fragments can be isolated from the aspirated sample and can be fixed and sectioned or be examined unstained for their fat content and hemosiderin granules In patients with iron deficiency hemosiderin is absent or present in only small amounts It is thought by many that smears from these spicules offer a more accurate estimate of the cellular content and arrangement of the bone marrow than smears from the total aspirate The differentiation between hyperplasia and hypoplasia of the bone marrow can be estimated by the total number of nucleated cells in the counting chamber or by inspection of the smear

Myeloid Erythroid Ratio The myeloid erythroid ratio provides an index of depression or hyperactivity of the granulocytic elements as compared with nucleated red cells In the newborn infant the M E ratio rises form 2 1 on the first day of life to about 10 1 or 12 1 in the second week indicative of granulocytic hyperplasia and a decline of erythrocyte production Beyond infancy the M E ratio is 2 5 to 3 5 1[14]

Roentgenographic Examination The greater value of the roentgenogram in the diagnosis of blood dyscrasias in earlier life than in later childhood and adult life can be related to the developmental features of the bone marrow The fact that all the bones are filled with red marrow is advantageous to the infant and young child in whom the demand for erythropoiesis is so active Fat appears in substantial amounts in the long bones at about 7 years of age Only with the appearance of nonfunctioning yellow marrow in the older child and its extension in the young adult is a potential reservoir available for the emergency formation of blood In the absence of yellow marrow the infant or young child faced with the need for excessive blood formation reactivates extramedullary fetal sites In addition the marrow hypertrophies and expands by absorption and atrophy of the bony trabeculae and the cortex These changes are observed in the roentgenograms and are of value in diagnosis More detailed discussions of the changes observed by roentgenography are described in connection with the specific blood disorders in this book and in the book by Caffey [1]

Following is a list of the bone and joint manifestations of the blood disorders of infancy and childhood

Disorders affecting blood cells and bone marrow

1 Red cells
   A Aplastic anemia (Fanconi syndrome)
   B Hereditary hemolytic anemias
      (1) Cooley s (thalassemia) anemia
      (2) Hereditary spherocytosis
      (3) Sickle cell anemia
2 White cell
   A Leukemia
3 Bone marrow
   A Marble bone disease
   B Myelofibrosis

Coagulation disorders

1 Classical hemophilia (antihemophilic globulin deficiency)
2 Christmas disease (plasma thromboplastin component deficiency)

Hereditary hemolytic anemias (severe Cooley s anemia sickle cell anemia hereditary spherocytosis)

Common features

1 Blood increased hemolysis and blood production
2 Bone changes due to compensatory marrow hypertrophy
3 X ray changes in skeleton including skull and vertebrae
4 Bone changes most marked in severe Cooley s anemia and least in spherocytic anemia

A *Cooley s anemia*
   1 Skeletal changes confined to severe cases
      (A) Dilatation of marrow cavities
      (B) Pressure atrophy of spongiosa and corticalis
         (1) Concave surfaces of metacarpals and long bones change to rectangular shape
         (2) Osteoporosis changing to sclerosis with age
         (3) Maturation and growth retarded
         (4) Pathologic fractures

2 Skull changes
  (A) Widening of diploic space hair on end appearance (also reported in patients with chronic iron deficiency anemia)
  (B) Swelling of zygomas
3 Vertebral changes reticulated occasionally compressed

B *Sickle cell anemia*
1 Marrow hyperplasia less marked than in Cooley s anemia
2 Skull changes as in Cooley s anemia
3 Long bones in children slight to no changes in adults fibrosis and thickening of cortex
4 Aseptic necrosis usually in femoral head less common in humeral head
  (A) Destructive and productive changes due to capillary thromboses and infarction
  (B) Occurs in sickle cell anemia sickle-hemoglobin C disease and sickle cell-thalassemia disease
5 Tendency toward osteomyelitis in salmonella infection
6 Vertebrae—compression deformity

C *Hereditary spherocytosis*
1 Skeletal and cranial changes as in Cooley s anemia but infrequent and less marked cranial changes more prominent than skeletal

Fanconi syndrome
1 Aplastic anemia in combination with congenital defects
  (A) Affecting heart skin mentality and genital development
  (B) Skeletal—variable absence of thumb calcaneal bones radius syndactylism

Osteopetrosis (marble bone disease Albers Schonberg disease)
1 Cause—failure to resorb calcified cartilaginous matrix
2 Increased thickness and density of the cortical and spongy portions of the entire osseous system bones brittle
3 X ray—individual bones and skull opaque heavy and lacking in finer structure
4 Tendency toward fractures slipping of epiphyses

Leukemia
1 Skeletal changes from encroachment upon functioning marrow by leukoblastic cells observed in metacarpals long bones and pelvis
2 Destruction of spongiosa erosion of cortex lifting of periosteum
  Osteoporosis cystic rarefaction moth eaten appearance of long bones
3 Transverse zones of diminished density in metaphyses of long bones

Hemophilia
1 Skeletal lesions due to
  A Bleeding directly into the bones
    (1) Shafts and epiphyses
      (a) Rounded defects in spongiosa
      (b) Cystic areas of rarefaction
    (2) Subchondral
      (a) Marginal bony defects at juxta articular borders—flattening deformity of proximal epiphyses of femur (like coxa plana)
    (3) Subperiosteal hematomas
      (a) Atrophy of underlying cortex
  B Bleeding into joint spaces
    (1) Retained blood and clots
      (a) Inflammation deformities and ankyloses destruction of articular cartilage thickened synovial membrane connective tissue invasion into joints
      (b) Generalized rarefaction of epiphyses and shafts

(2) Regional hyperemia and recurrent hemarthroses result in hypertrophy of adjacent epiphyses

C Hemarthroses—marked in both classical hemophilia (antihemophilic globulin deficiency) and in Christmas disease (plasma thromboplastin component deficiency)

Hereditary Factors as an Aid in Diagnosis The genetic aspects of disease are manifested in a variety of hematologic disorders notably erythroblastosis fetalis the hereditary hemoglobinopathies the group of primaquine sensitive hemolytic anemias hereditary spherocytosis and elliptocytosis and the coagulation defects Specific genetic implications are discussed in connection with each entity elsewhere in the book The discovery of hereditary factors depends upon the application of selected laboratory tests made not only on the affected patient but also on his asymptomatic relatives for the signs of the trait Tests in common use include examination of the blood smear for morphologic abnormalities fragility tests serologic techniques starch and paper electrophoresis exposure of red cells to specific chemical agents to determine their tendency toward hemolysis coagulation studies and a comprehensive investigation of the family for incidence of the disease An analysis of the data provides insight into the genetic control of these disorders extending to their qualitative representation

Thalassemia sickle cell anemia and hereditary spherocytosis may be cited as examples of familial diseases in which the hereditary trait may be recognized by suitable blood studies Their hereditary nature is reflected in the relatively high incidence in members of the same family The diagnosis of these diseases in a child with an obscure anemia of moderate severity often can be made earlier in its course and useless therapy avoided by detection of the trait in the parents and siblings In the patient with thalassemia for instance the most important element in the diagnosis of the milder form with the simple means immediately available to the practitioner is the discovery of qualitatively similar alterations in the blood of other members of the same family These persons are asymptomatic and have either mild anemia or no anemia Regardless of the hemoglobin level their blood shows hypochromic macrocytes stippled erythrocytes polycythemia increased resistance of the red cells to hemolysis in hypotonic solutions of sodium chloride and less frequently target or oval cell The evidence is conclusive that in every family with a child having thalassemia major requiring periodic transfusions both parents reveal evidence of the disease When this bilateral inheritance of the gene for thalassemia is nonexistent a search is made by electrophoresis and sickle cell preparations for the presence of another abnormal hemoglobin in the seemingly unaffected parent

Diagnostic Features of the Blood Smear Of all laboratory procedures the most important for its diagnostic value yet the simplest is the examination of the blood smear Although corroborative evidence from auxiliary sources may be required to establish a final diagnosis the stained blood film constitutes a visual representation of the effect on morphology of the factors involved in the pathogenesis of a specific anemia In describing the changes in the red blood cells it should be pointed out that except for sickle cells spherocytes and leptocytes (the large pale extremely thin ridged erythrocytes with irregularly distributed hemoglobin observed in the blood of patients with thalassemia major) there are no specific red cells that are indicative of a particular disorder Even the small deeply stained spherocytes which characterize congenital spherocytic anemia may also be observed in greater or lesser degree in patients with other conditions associated with hemolysis such as acquired hemolytic anemia erythroblastosis due to sensitization by the A B agglutinogens and leukemia Target cells oval cells

hypochromic macrocytes and basophilic stippling and hypochromic microcytes appear in varying percentage in certain stages of many anemias and therefore cannot be regarded as distinguishing features of a single disease. They are of

*Table 8 Diagnostic Features of the Blood Smear in the Anemias of Early Infancy**

Type of Anemia	*Diagnostic Features*
Erythroblastosis fetalis	Hyperchromic macrocytes nucleated red blood cells
Hemorrhagic anemia	Acute blood loss normochromic and normocytic red blood cells Chronic blood loss hypochromic microcytes
Iron deficiency anemia	Hypochromic microcytes marked central pallor of red blood cells poikilocytes
Anemia of prematurity	Early—normocytic and normochromic red blood cells later—hypochromic microcytes
Megaloblastic anemia of infancy	Unusually large macrocytes microcytes occasional nucleated red blood cells abnormal neutrophils

From Smith C H Anemias in Infancy and Childhood Diagnostic and Therapeutic Considerations Bull New York Acad Med 30 155 1954

*Table 9 Diagnostic Features of the Blood Smear in the Anemias of Later Infancy and Childhood**

Type of Anemia	*Diagnostic Features*
Anemia of infection	Normocytic and normochromic occasionally microcytic and hypochromic red blood cells with iron deficiency hypochromic microcytes predominate
Aplastic hypoplastic and aregenerative (pure red cell) anemia	Normochromic and normocytic red blood cells
Acquired hemolytic anemia	Moderate spherocytosis marked polychromasia reticulocytosis
Congenital nonspherocytic hemolytic anemia (may appear at birth)	Normochromic and normocytic reticulocytosis occasionally macrocytosis anisocytosis and poikilocytosis
Hereditary spherocytosis (may appear at birth)	Spherocytes polychromasia reticulocytosis
Sickle cell anemia	Sickle cells target cells microcytes occasional nucleated red cells
Thalassemia major (Cooley's anemia)	Very large thin hypochromic macrocytes nucleated red blood cell marked poikilocytosis anisocytosis
Thalassemia minor	Hypochromic macrocytes microcytes basophilic stippling target and oval cells

*From Smith C H Anemias in Infancy and Childhood Diagnostic and Therapeutic Considerations Bull New York Acad Med 30 155 1954

diagnostic value only when they are considered in conjunction with pertinent information from other sources The morphologic changes of the red cells in the most common anemias of infancy and childhood are summarized in Tables 8 and 9 Each of these disorders is described in greater detail elsewhere in the book

Principles of Treatment The information gathered thus far has provided a sufficiently firm foundation with respect to pathogenesis to warrant a consideration of therapy In Table 10 are listed the available agents in current use designed to correct an underlying deficiency or to ameliorate the disorder by accessory means when direct remedies are not available The constant emphasis on the use of specific rather than indiscriminate mixtures of antianemia substances[11] applies with especial force to the pediatric age group Iron salts given orally preferably in the ferrous forms without the aid of supplementary minerals or vitamins still constitute the most effective treatment of iron deficiency anemia Treatment with iron salts in patients with other conditions may not only be futile but also potentially harmful The anemias for which transfusions are indicated consist of two groups those in which this measure constitutes a temporary expedient until spontaneous recovery sets in and those in which in the absence of specific therapy the need for restoration of optimum blood levels is continuous or urgent The indications for splenectomy will be discussed in Chapter 25

Allergic Implications of Blood Disorders The clinical manifestations of many of the blood disorders are wholly or partially dependent upon immunoallergic reactions The type of response resulting from the combination of an antigen with an antibody has its counterpart in the immunoallergic mechanisms underlying many of the blood disorders Many of these are now classified under the heading

Table 10 Therapy of the Common Anemias of Infancy and Childhood

Agent	*Type of Anemia*
Iron	Iron deficiency anemia late anemia of prematurity chronic blood loss
Folic acid—ascorbic acid(?) vitamin B_{12}(?)	Megaloblastic anemia of infancy
Transfusion	Erythroblastosis nonhemolytic anemia of newborn infant anemia due to hemorrhage and infection aplastic hypoplastic and pure red cell (chronic congenital aregenerative) anemias
Splenectomy	Spherocytic anemia acquired hemolytic anemia in selected cases thalassemia major sickle cell anemia aplastic anemia hypoplastic anemia and pure red cell anemia
ACTH and steroids (including testosterone)	Acquired hemolytic anemia aplastic hypoplastic and pure red cell anemia (?) crises of sickle cell anemia (?)

From Smith C H Anemias in Infancy and Childhood Diagnostic and Therapeutic Considerations Bull New York Acad Med 30 155 1954

of immunohematology and include the hemolytic anemias granulocytopenia and thrombocytopenia The pathogenesis is based to some extent on antibodies to the respective formed blood elements blood vessel hypersensitivity incompatibility between blood elements of fetus and mother transfusion reactions and sensitivity induced by bacterial chemical and physical agents[9] Hypersensitivity to drugs may result in hypoplastic or aplastic anemia in contrast to primaquine sensitive hemolytic anemias which are based on an inherited intrinsic defect of the red cells

It is of interest to consider the extraordinary phenomenon by which antibodies which are capable of reacting with the patient s own red cells are elaborated A similar situation occurs in those patients with thrombocytopenic purpura and agranulocytosis in whom antibodies are directed against the patient s platelets and polymorphonuclear cells These disorders call attention to the remarkable tolerance developed from early fetal life by which the body in health avoids forming antibodies against its own tissues Such dire circumstances in which a living organism becomes capable of producing antibodies against its own body constituents was termed horror autotoxicus by Ehrlich[13] The fundamental feature of autoimmune acquired hemolytic anemia and other disorders characterized by autoimmune antibodies to blood cell elements is the loss of this normally acquired tolerance with the development of antibodies to one s own cellular antigens

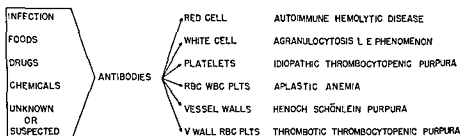


Fig 5 Immunoallergic concept of blood disorders (From Smith C H Allergic Implications of Blood Disorders in Infancy and Childhood California Med 86 366 1957)

Once the effects of the hypersensitive state are set in motion during the course of a blood disorder therapeutic measures to slow their progress are often futile Because it is not always possible to identify the potentially allergic child in whom these circumstances will occur it is extremely important to weigh the advantages of the use of a drug before it is administered especially when its side effects have not yet been thoroughly investigated

Following is a list of blood disorders in infancy and childhood many of whose clinical and laboratory features lend themselves to an interpretation on an immunoallergic basis (also see Fig 5)

Disorders affecting blood cell and vascular elements

1 Red cells
 A Acquired hemolytic anemia
  (1) Toxic products drugs favism

From Smith C H Allergic Implications of Blood Disorders in Infancy and Childhood California Med 86 366 1957

(2) Antibodies against red cells
(a) Erythroblastosis fetalis
(b) Blood transfusion reactions
(c) Associated with autoantibodies primary autoimmune hemolytic anemia secondary leukemia Gaucher s disease
(d) Pure red cell (chronic congenital aregenerative) anemia
2 White blood cells
A Agranulocytosis
(1) Anticonvulsants
(2) Antimicrobial agents
(3) Antithyroid drugs
(4) Sulfonamides
B Neonatal agranulocytosis
3 Platelets
A Thrombocytopenic purpura
(1) Idiopathic
(2) Congenital
(3) Drug induced quinine quinidine sulfonamides arsenicals
(4) Eczema with purpura and otitis media
4 Red cells white cells and platelets
A Hypoplastic and aplastic anemia
(1) Idiopathic
(2) Drugs chemical infection
5 Blood vessels
A Allergic purpura (Henoch Schonlein syndrome)
B Idiopathic pulmonary hemosiderosis
C Polyarteritis nodosa and other collagen di eases
D Thrombotic thrombocytopenic purpura (vascular vessel wall in combination with platelets and red cells)

Infections
1 Infectious mononucleosis
2 Susceptibility following splenectomy

Autoerythrocytic Sensitization The symptoms in this condition are easy bruising with swelling edema, and painful ecchymoses due to autosensitivity of patients to their own blood Severe trauma with bruising characterize the history A stromal factor of red cells may be the responsible agent for the clinical manifestations and for the positive skin tests Extremely painful reactions follow intradermal testing with red cells This syndrome corresponds to the type of autosensitization noted in patients with lupus erythematosus some forms of acquired hemolytic anemia and thrombocytopenic purpura [4]

REFERENCES

1 Caffey J Pediatric X ray Diagnosis Chicago 1956 Year Book Publishers Inc
2 Dacie J V Autoimmunization in Respect of Red Cells Program of Sixth International Congress International Society of Hematology Boston 1956 p 488
3 Gaffney P C Hausman C F and Fetterman G H Experience With Smears of Aspirates From Bone Marrow in the Diagnosis of Neuroblastoma, Am J Clin Path 31 213 1959
4 Gardner F H and Diamond L K Autoerythrocytic Sensitization a Form of Purpura Producing Painful Bruising Following Auto Sensitization to Red Blood Cells in Certain Women Blood 10 675 1955

5 Kato K and Wachter H F Adrenal Sympathicoblastoma in Children With Special Reference to the Biopsy of Sternal Marrow and of Metastatic Nodule in the Skull J Pediat 12 449 1938
6 Kirkman H N and Riley H D Posthemorrhagic Anemia and Shock in the Newborn a Review Pediatrics 24 97 1959
7 Price Jones C Red Blood Cell Diameters London 1933 Oxford Medical Publications
8 Smith C H Bone Marrow Examination in Blood Disorders of Infants and Children M Clin North America 31 527 1947
9 Smith C H Allergic Implications of Blood Disorders in Infancy and Childhood, California Med 86 366 1957
10 Whitby L E H and Britton C J C Disorders of the Blood ed 8 New York 1957 Grune & Stratton Inc
11 Wintrobe M M Editorial Shotgun Antianemic Therapy Am J Med 15 142 1953
12 Wintrobe M M Clinical Hematology ed 4 Philadelphia 1956 Lea & Febiger
13 Witebsky E Ehrlich s Side Chain Theory in the Light of Present Immunology Ann New York Acad Sc 59 168 1954
14 Wolman I Laboratory Applications in Clinical Pediatrics New York 1957 Blakiston Co

CHAPTER 12

Iron-Deficiency Anemia

Iron deficiency anemia represents the most common nutritional deficiency in children and is especially prevalent in infancy. The unusual susceptibility of the infant is due to a variety of interrelated factors inherent in this age period which affect iron metabolism.

Etiology. Iron deficiency anemia occurs most commonly between the ages of 6 and 24 months and reflects either an inadequate supply or an excessive demand for iron. These factors are interdependent and overlap. In the first six months the infant is dependent upon iron reserves acquired during fetal life. With the depletion of these stores the infant requires dietary iron to meet the demands of growth. The period before and after 6 months of age cannot be sharply circumscribed because of the individual variability of iron stores and the extent of growth. The major etiologic factors in iron deficiency in infancy and childhood are as follows:

1. *Inadequate supply of iron*
   - A. Inadequate iron stores at birth
     - (1) Premature, twin, or multiple births
     - (2) Severe iron deficiency in the mother
     - (3) Fetal blood loss at or before delivery
       - (a) Fetal hemorrhage into maternal circulation
       - (b) Retroplacental bleeding
   - B. Inadequate intake—deficient dietary iron
2. *Impaired absorption*
   - A. Chronic diarrhea
   - B. Celiac disease
   - C. Gastrointestinal abnormalities
3. *Excessive demands for iron*
   - A. Blood loss during infancy
     - (1) Acute or chronic hemorrhage
     - (2) Parasitic infection such as hookworm
   - B. Failure to meet increased demands for growth
     - (1) In the premature infant and in other rapidly growing infants
     - (2) During adolescence

In a series of 272 infants whose blood was found to contain less than 9 gm of hemoglobin per 100 ml, Woodruff[8] found that the most common cause of

hypochromic anemia was prematurity or a birth weight of less than 3 kg The incidence of severe hypochromic anemia in infants weighing more than 4 kg was extremely low A first child was less likely to become anemic than were later siblings single children were less likely than twins and girls less likely than boys[8] Guest and Brown[6] also found a significantly lower incidence of iron deficiency anemia in first born infants than later born infants

Prenatal factors play an important role in influencing the iron content of the fetus These include the length of gestation maternal hemoglobin concentration and less frequently occult or frank hemorrhage from the fetal surface of the placenta at or before delivery Abnormal placental circulation in multiple births especially in single ovum twins may result in the transfusion of one twin by another[36] Such transfusions may explain the relatively frequent occurrence of hypochromic anemia in only one twin regardless of similar growth rates and diets[8]

The only source of iron for the fetus is derived transplacentally from the maternal organism To provide for increased needs incident to the rapid growth of the fetus in the last trimester of pregnancy iron absorption in the mother is accelerated [48]

The average adult has about 3 to 5 gm of iron in his body of which 2 to 3 gm are found in the hemoglobin 1 to 1 5 gm in the body stores as ferritin and hemosiderin and the rest in myoglobin respiratory enzymes and the plasma The cytochromes are iron-containing porphyrin compounds which function as respiratory enzymes in the cell In contrast at birth the infant s supply is approximately 300 mg provided by the mother after birth iron for the increasing needs of the growing body is derived from the diet

Although the iron transfer to the fetus is negligible during the first two trimesters of preg nancy it rises in the third trimester to 4 mg daily so that the total amount accumulated by the fetus at term approximates 300 mg Widdowson and Spray found an average of 273 mg of iron in six infants weighing more than 3 000 gm They found that the iron concentration *during fetal life was relatively constant with an average value of 75 mg per kilogram* of body weight Of this the liver and spleen contained one eighth of the total (34 mg) The bulk of iron available to the infant at birth for eventual hemoglobin formation is that con tained in the circulating hemoglobin Josephs[illegible] *estimated* approximately 30 to 35 mg in the liver The amount of the nonhemoglobin iron fraction (myohemoglobin cytochromes and other respiratory enzymes) at birth has been variously estimated at 4 mg per kilogram[illegible] 6 mg per kilogram[illegible] and 7 5 per kilogram The total hemoglobin mass may be computed by mul tiplying the *blood* volume of 85 ml per kilogram (variation 80 to 90 ml per kilogram) by the hemoglobin concentration per 100 ml of blood Since each gram of hemoglobin contains 3 4 mg of iron the total hemoglobin value is multiplied by this figure to convert it to total *body iron With these values Sturgeon calculated*[illegible] *in a hypothetical infant weighing* 4 kg that 232 mg of iron is present in circulating hemoglobin 51 mg in the liver and spleen and 16 mg as myoglobin and other parenchymal iron In an infant weighing 3 kg at birth the figures accordingly would be 106 mg from circulating hemoglobin 11 mg in stores and 12 mg in nonhemoglobin iron

Premature twin and multiple births reduce the quantity of iron in the storage depots These factors also tend to reduce the total amount of circulating hemo globin and therefore the content of iron required for later hemoglobin synthesis Less frequently reduced stores result from hemorrhage due to loss of fetal blood into maternal circulation[8] from placenta previa from abruptio placentae and

from injury to the cord during delivery Accidental bleeding from the cord following delivery reduces the initial iron stores in relation to the extent of hemorrhage Early or late clamping of the cord is another important factor contributing to the iron endowment of the infant Manual stripping of the cord can add as much as 75 ml of whole blood or 40 mg of iron to the storage depots [67] It has been emphasized [6] that the technique of delayed ligation of the cord does not result in a significant increase in red cell volume unless the cord is initially stripped or milked

Full term infants born to nonanemic mothers receive sufficient iron during fetal life to meet their needs for at least the first three to four months of infancy [81, 8] With few exceptions infants born to mothers who have suffered iron depletion during pregnancy show normal hemoglobin concentrations at birth The influence of an inadequate iron endowment does not become manifest until later in the first year In markedly anemic mothers administration of iron before delivery of their babies prevents the development of anemia in the newborn infant [60, 6] Unless the anemia in the mother is marked (below 9 gm per cent) iron deficiency usually does not occur in the infant [81] Despite these facts maternal iron depletion as indicated by severe anemia is not regarded as a common predisposing factor of iron deficiency in infants in the United States at the present time [1] Administration of iron supplements to normal mothers overcomes the mild physiologic anemia of pregnancy but exerts little if any influence on the offspring s iron endowment at birth or iron nutrition in infancy [68, 69]

Iron deficiency anemia in the full term infant which is noted at approximately 6 months of age cannot be evaluated properly unless important hematologic events in the preceding period are considered These two periods are not sharply circumscribed but one merges with the other since both are affected by forces within and without the hematopoietic system Of basic importance is the fact that the iron depots at birth possessing variable amounts of this mineral (less in the premature and more in the full term infant) are substantially increased by progressive accumulation of iron in the first months of life [40] This major iron supplement is derived from the degradation of circulating hemoglobin mass with which the infant is born Regardless of the amount the initial iron tissue stores at birth cannot be underestimated for their value in hemoglobin synthesis

Relation of Physiologic Anemia of the Newborn Infant to Iron Deficiency Anemia Following an initial week or ten days of stable or mildly fluctuating blood levels the hemoglobin and red cells drop reaching a minimum at approximately 7 weeks of age in the premature infant and at 2 to 3 months in the full term infant This phase designated as physiologic anemia of the newborn infant is highly important in the iron economy of the infant since the iron liberated by red cell destruction is stored for later use in rebuilding hemoglobin During this period the red cell count falls proportionately to hemoglobin so that a normochromic normocytic anemia develops It should be emphasized that the anemia at this stage is due to a depression of erythropoiesis and hemoglobin synthesis during which red cells are destroyed at normal or at best slightly increased rates [4]

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apical systolic murmur is frequently heard but the heart is not enlarged The changes in the epithelial structures characterizing iron deficiency in adults such as atrophic glossitis dysphagia and spoon shaped or concave nails (koilonychia) *do not occur in infants and are only rarely observed in older children* Because of gradual development of the anemia signs of cardiac dysfunction are usually not encountered even in infants with severe grades of hemoglobin reduction

Laboratory Data *Important laboratory information is obtained from a study* of the peripheral blood bone marrow and plasma of patients with iron deficiency anemia

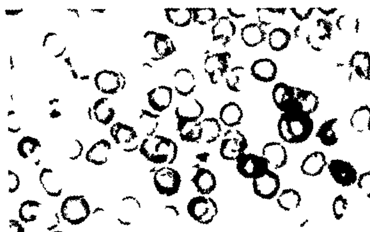


Fig 6 Blood smear of an infant with iron-deficiency anemia Note predominance of hypochromic microcytes fairly uniform in size and shape whose outstanding feature is marked deficiency in hemoglobin indicated by central pallor (× 1350) (From Smith C J Treatment of the Anemias of Infancy and Childhood J Pediat 32 721 1948)

Blood The anemia of iron deficiency is characteristically hypochromic and microcytic in contrast to the normochromic normocytic type of physiologic anemia in the postnatal period Since iron deficiency *primarily affects hemoglobin* synthesis and red cell formation to a lesser degree the red cell count may be normal near normal or moderately reduced The hematocrit levels are lower than normal The decreased hemoglobin content of the individual erythrocyte is the specific abnormality present in iron deficiency anemia The hemoglobin is reduced out of proportion to the red cell count The red cell count varies usually ranging from 3 to 5 million with a hemoglobin level from 3 to 9 gm per 100 ml The following criteria of hypochromic anemia may be designated hemoglobin *concentrations below 9 gm per 100 ml a mean corpuscular hemoglobin* (MCH) less than 27 micrograms a mean corpuscular hemoglobin concentration (MCHC) of less than 30 per cent[8] and a mean corpuscular volume (MCV) of less than 78 cubic microns[1]

The blood smear shows microcytes poikilocytes elliptical and elongated pencil forms and target cells Many of the red cells are diminutive in size A small

The diminished hematopoiesis is explained by Gairdner and associates[18] as an adjustment to the changeover from the placenta to the lung as a source of oxygen. To maintain a normal postnatal oxyhemoglobin content of the blood there is no need for elevated hemoglobin levels so that erythrogenesis and hemoglobin synthesis are retarded until such a time as the hemoglobin level drops from elevated blood levels at birth to approximately 11 to 12 gm. per 100 ml. Any decrease below this point induces increased hematopoietic activity, whereas elevations above this level will tend to decrease marrow activity. When the designated concentrations are reached in the full term infant at 2 to 3 months of age, marrow erythroid activity is resumed[18] as reflected by reticulocytosis and the gradual rise in the circulating hemoglobin mass. A fundamental principle in the kinetics of iron metabolism is that once iron is introduced into the body it is used again and again. The amount excreted at any one time is small and is assumed to be less than 1 mg. daily.

At approximately 16 weeks of age in the full term infant the total amount of circulating hemoglobin is restored to the initial birth values. A humoral erythropoietic stimulating factor (erythroprotein)[1] may also be involved in these fluctuations, functioning when anemia develops and being inactive at the high hemoglobin concentrations at birth.

With the resumption of active erythropoiesis, reticulocytes appear and the circulating mass expands. In both full term and premature infants the amount of hemoglobin synthesized will be limited by the iron which has accumulated in the stores during the postnatal drop in hemoglobin. This in turn depends principally upon hemoglobin concentration and hemoglobin mass available at birth and supplemented by a variable component of pre-existing iron in the liver and spleen. Increase in weight and the blood volume obscures the improvement in the hemoglobin concentration during the period of recovery.

The supply of iron becomes strained with rapid growth so that the infant is exposed to an anemia based on the exhaustion of storage iron. Following the period of reutilization of iron and hemoglobin synthesis and increase in total hemoglobin mass, *the supply of this mineral becomes depleted unless the stores* are replenished by exogenous iron from an adequate diet. These demands are met in the average full term infant. The failure to add foods containing significant quantities of iron leads to iron deficiency anemia, especially if the demands are excessive. Iron deficiency anemia accordingly is observed in clinical practice in infants who are growing rapidly and are fed almost exclusively on diets of milk and cereals with low iron content and present feeding difficulties.

Clinical Features. Infection, which plays a dominant role as an etiologic factor in anemia in infancy and childhood, frequently highlights an underlying state of iron deficiency. However, pallor, irritability, anorexia, listlessness and gastrointestinal disturbances usually direct attention to severe grades of this disorder. A history of repeated upper respiratory *difficulties is not uncommon.*

In no condition is the sheetlike or waxy pallor so striking as in advanced iron deficiency anemia. Except for pallor of the skin and mucous membranes and occasionally a moderately enlarged spleen, the patients present no significant physical abnormalities. As in patients with other severe anemias, a soft blowing

microcytic picture and other diagnostic aids are necessary. The recent discovery of *increased levels of A hemoglobin in the mild type of the disease* (thalassemia heterozygotes) has proved to be of confirmatory value.[38]

Confusion with the severe homozygous type of Cooley's anemia may occur only in the infant in the first year of life. The large pale extremely thin erythrocytes with irregularly distributed hemoglobin and scattered normoblasts interspersed among microcytes in the blood smear are in contrast with the more uniform microcytes of iron deficiency anemia. Occasional hypochromic microcytes are, however, also found in the latter. The significant splenomegaly and the presence of the trait in both parents and siblings further differentiates Cooley's anemia from iron deficiency anemia.

Changes of the skull identical with the "hair standing on end" appearance observed on the roentgenogram of patients with the chronic hemolytic anemias *have been described in patients with chronic iron deficiency anemia, especially* in premature infants and twins.[4, 11, 58] These changes are associated with erythroid hyperplasia in the bone marrow. In contrast to changes observed in patients with Cooley's anemia, these changes are confined to the skull. The roentgenograms *of the long bones fail to reveal any abnormality.*

Treatment. It has been established clinically and in the laboratory that treatment with simple iron salts is effective in correcting iron deficiency anemia. Nevertheless, successful management requires a thorough investigation of basic *etiologic factors such as faulty diet, blood loss* and malabsorption due *to chronic* diarrhea or a structural gastrointestinal defect such as polyps or Meckel's diverticulum. Most commonly the history will reveal a dependence on foods notably poor in iron content such as milk, cereals and other carbohydrate foods. Often this situation will have developed unwittingly *from a failure of parents to under*stand the need for a well balanced diet and its particular importance in the rapidly growing infant. Each of these factors can be eliminated by diagnostic and therapeutic procedures and the diet can be adjusted. Restriction of milk to one pint a day, the introduction of meat, vegetables and fruit, and supplementation by an iron preparation usually will suffice to correct the anemia.

Of importance in all infants, the full term and especially the premature, is *the insistence upon serial hemoglobin determinations* from the second month and at appropriate intervals thereafter for the detection and control of the anemia. The necessity for this measurement stems from the variability in the hemoglobin mass at birth from which iron is derived for later hemoglobin synthesis and the individual needs for exogenous iron due *to growth of tissues* and *expanding* blood volume.

To achieve maximum improvement in hemoglobin concentration, Sturgeon[illegible] has suggested that the recommended daily allowance of 6 mg. of food iron through the first year and 10 mg. *from 1 to 3 years of age be increased to intakes of 6 to 9 mg. daily* at 3 months of age, to 8 to 12 mg. by 6 months, and to 10 to 15 mg. at 12 months. One egg yolk supplies approximately 1 mg. of iron, and peach, the best source of iron among the fruits, provides 0.3 mg. per tablespoon.[illegible]

Based on data from Food and Nutrition Board of the National Research Council.

number of normal sized red cells and microcytes are usually present, often with a normal hemoglobin content. The characteristic appearance of the blood smear is the presence of a majority of small red cells with marked central pallor with fine and poorly staining rims of hemoglobin. Occasional stippled red cells and normoblasts may be present. Reticulocytes are normal or reduced. The fragility of the red cells may be normal or may show a moderate resistance to hemolysis in hypotonic solution of sodium chloride. The platelet count is normal. The leukocytes are usually normal or slightly reduced.

Bone Marrow. The bone marrow shows erythroid hyperplasia with a predominance of polychromatophilic normoblasts, often smaller than normal. The cytoplasm may be diminished to the extent that it forms a small rim around the nucleus. Granulopoiesis is normal. Megakaryocytes are normal in number and in appearance. Contributory evidence of diminished iron stores may be directly determined by bone marrow examination which reveals a decrease of iron granules in the normoblasts (sideroblasts)[10,34] and an almost complete absence of stainable iron or hemosiderin in marrow aspiration. This depletion of hemosiderin is contrasted with the increased quantities noted in infection and in hemolytic anemias.[31,64]

Plasma. The serum iron is reduced, varying from 10 to 60 micrograms per 100 ml. and averaging about 30 micrograms per 100 ml. (normal approximately 120 micrograms). The latent iron binding capacity of the serum is increased to approximately 450 micrograms and above (normal 250 micrograms per 100 ml.). The saturation of serum iron (serum iron divided by serum iron plus latent iron binding capacity) is reduced and averages 6 per cent[61] (normal 32 per cent). A greatly reduced serum iron and a markedly elevated latent iron binding capacity have not been found in any other condition than iron deficiency and are therefore of diagnostic value. With treatment the serum iron is increased, the latent iron binding capacity undergoes a moderate contraction and serum iron saturation increases. In addition to hypoferremia and reduced saturation of total serum iron, other chemical manifestations of iron deficiency include increased blood copper levels (hypercupremia) and increased free erythrocyte protoporphyrin.[66]

Of the many diagnostic features delineating the hematologic picture of an anemia due to a primary deficiency of iron, the simplest is the presence of microcytic hypochromic red cells which dominate the stained blood smear.

Diagnosis. The characteristic small pale red blood cells of iron deficiency anemia in the stained smear, a history of an iron deficient diet and the marked pallor of the patient are usually sufficient to make the diagnosis in the infant over 6 months of age. The stool examination for unrecognized chronic blood loss is essential.

Iron deficiency anemia and milder forms of thalassemia are often indistinguishable. The red cells in both conditions show moderate stippling and target and oval forms, but the larger number of hypochromic macrocytes in the blood smear and the familial hereditary pattern are important diagnostic features of thalassemia minor. The blood smear of patients with mild thalassemia and of those with iron deficiency may both show, however, a more or less uniform hypochromic

salts or because it shares with other foods of increased phosphorus content a basic difficulty in absorbing iron [8] Phytates contained in cereals also make non ionized precipitates with iron Despite these findings iron salts in therapeutic doses added to milk however have produced satisfactory hemoglobin responses in our experience and in that of others [10] Furthermore the iron in iron supplemented cereals now in common use in infant feeding is also well absorbed

Oral administration of iron usually results in the stool becoming a deep black color due to the increased content of iron sulfides The absence of this change may serve as a clue to the irregular administration of the iron [6] Ingestion of liquid iron preparations may produce a black staining of the teeth Brushing the teeth after each administration is of value in reducing this untoward effect although the staining is only temporary

For older children tablets of ferrous sulfate or ferrous gluconate are preferable to the concentrated liquid preparations used with infants Gastric irritation nausea vomiting and abdominal pain are less likely to occur if the tablets are taken with meals Iron tablets are best taken three time daily at mealtime Tablets of ferrous sulfate (0 2 gm 3 grains) or ferrous gluconate (0 3 gm 5 grains) given three times daily provide a daily total of 100 to 200 mg of elemental iron

In the infant as in the older child full therapeutic doses of iron should be given for at least six to eight weeks after the hemoglobin has been restored to normal levels If oral therapy is withdrawn too soon the iron stores will remain unreplenished [61] and anemia will eventually recur

Iron dextran for intramuscular use Parenteral iron therapy is indicated when patients fail to respond to adequate oral dosage because of gastrointestinal irritation gastrointestinal pathology or more rarely refractoriness to oral therapy because of failure to absorb iron An iron dextran mixture for intramuscular use is now available (Imferon) which has proved a valuable adjunct to therapy This preparation is well tolerated and provides 50 mg of elemental iron in each milliliter The total amount of iron needed to raise the hemoglobin level to normal and replenish stores is calculated as follows

$$\frac{\text{Normal hemoglobin} - \text{Initial hemoglobin}}{100} \times \text{Blood volume (milliliters)} \times 3\,4 \times 1\,5$$

1 Normal hemoglobin = 11 to 12 gm in infants 12.5 to 13.5 gm in children 14 to 15 gm at puberty
2 Blood volume = 80 ml per kilogram or 40 ml per pound of body weight
3 3 4 converts grams of hemoglobin into milligrams of iron
4 Factor 1 5 provides extra iron to replace depleted tissue stores

Example Infant weighing 10 kg with 5 gm of hemoglobin per 100 ml

Blood volume = 80 ml per kilogram × 10 kg = 800 ml

Hemoglobin deficit = 12 gm (approximately normal for age) − 5 gm = 7 gm per 100 ml

Total hemoglobin deficit = 7 gm × $\frac{800}{100}$ = 56 gm

Iron to restore hemoglobin to normal = 56 gm × 3 4 mg = 190 mg
Iron to replenish stores = 50 per cent of 190 mg = 95 mg
Total dose of iron = 190 + 95 = 285 mg of iron

At present Imferon has been temporarily withdrawn from the market

Iron rich foods include the following commercially prepared dry ready to serve infants cereals (0 92 mg of iron per tablespoon) green and yellow vegetables (0 05 mg per table spoon in spinach) green beans (0 28 mg per tablespoon) and liver (0 56 mg per tablespoon)

Josephs gives the iron content of milk as follows As it comes from the cow it contains about 0 4 to 0 5 mg of iron per liter Commercial pasteurization increases the iron to 0 7 to 1 0 mg per liter Processed milk whether powdered or condensed may contain 1 to 2 mg per liter Breast milk contains 0 7 to 1 0 mg per liter

Iron Therapy Treatment with a soluble iron salt preferably ferrous iron corrects the deficiency more promptly than the ingestion of foods rich in iron although both should be given simultaneously Ferrous iron permits significantly higher hemoglobin values in well children than does ferric iron of the same dosage[46] Ingested iron is absorbed in the ferrous form largely from the duodenum Ferrous sulfate is the preparation of choice in most infants because the metallic iron content is adequate and it is efficiently absorbed inexpensive and easily administered Normal infants will absorb an average of 10 to 20 per cent of orally administered ferrous sulfate in solution and iron deficient children absorb 5 to 10 per cent more [6]

Normal children absorb an average of about 10 per cent of the naturally occurring iron in milk eggs chicken liver and iron supplements added to commercially prepared infants cereals[55] Iron deficient children absorb 2 to 3 times as much food iron as do normal children[56] In normal adult subjects on the contrary approximately 5 to 15 per cent absorption takes place from ferrous salt given in 100 mg amounts[18] With uncomplicated iron deficiency anemia the absorption is increased to between 25 and 70 per cent With food iron the percentage of absorption is 1 to 10 per cent which is increased to 20 to 30 per cent in the iron deficient subjects Reducing substances especially ascorbic acid enhanced the absorption of food iron With nearly 5 to 10 per cent of iron in food assimilated by normal adults the daily retention on a diet containing 12 to 15 mg is approximately equivalent to the amount of iron lost from the body each day

DOSAGE AND MODE OF ADMINISTRATION Since the percentage of metallic iron content is a fraction of the entire compound iron should be prescribed with this fact in mind Such preparations are now available and are suitably designated according to iron content

For infants ferrous sulfate is available in concentrated solutions which can be given in drop dosage in which each drop contains a measured amount of elemental iron The total dose of elemental iron recommended for an infant during the period of iron deficiency (6 to 24 months) ranges from 60 to 90 mg Ideal treatment consists of the administration of such a soluble iron preparation in divided dosage preferably between meals

There are a large variety of iron salts ferrous and ferric alike available in solution form that produce a satisfactory hemoglobin response the ferric solutions require a somewhat higher dosage The pediatrician should have knowledge of several preparations with respect to details of administration and content of elemental iron since it is occasionally necessary to interchange them because of gastrointestinal irritation or anorexia produced by one or another preparation

Milk usually has been regarded at least theoretically as being an unfavorable vehicle for iron either because it combines with phosphates to form unsoluble

Cobalt has been advocated in the treatment of anemia because of the known development of polycythemia when it is fed to experimental animals. Plasma from animals injected with cobaltous chloride rapidly develops a high titer of erythropoietin. Cobalt is an essential element in vitamin B_{12} (cyanocobalamin). Despite these considerations and reported beneficial results in the treatment of anemia associated with renal disease, cobalt has been found lacking as a therapeutic agent in patients with iron-deficiency anemia, hypoplastic anemia, and other instances of bone marrow failure. Of even greater concern is its possible toxicity in some patients, producing a goitrogenic effect in young infants, hence the danger of recommending cobalt alone or cobalt with iron for clinical use.

Copper is normally found in the blood in both the plasma and the red corpuscles. Serum copper increases in pregnancy, infection, and iron deficiency. In adult males the concentration of copper in the serum is 105 micrograms per 100 ml with a standard deviation of ±16. In normal infants beyond 8 months of age the serum copper values are elevated. The range from 140 to 200 micrograms per 100 ml is evidence of iron depletion during infancy.

Experimental studies have shown that copper plays a role in the absorption and utilization of iron. With serious depletion of copper not only is iron absorption impaired but also erythropoiesis is decreased and the defective erythrocytes that are produced have a shortened survival.

In spite of the striking evidences of anemia in copper-deficient experimental animals, the usefulness of copper in the therapy of anemia in man has still to be demonstrated. Copper is known to exist as an impurity in the therapeutic iron salts. It is of interest that processed baby foods such as strained beef liver and cereals and, to a lesser extent, fruits and vegetables possess the highest level of copper. These foods are certainly sufficient to ensure the infant's daily requirement of copper of 0.05 mg per kilogram of body weight per day. The major portion of copper in the plasma is found in the A_2 globulin ceruloplasmin.

Transfusions With the availability of suitable iron preparations for oral and parenteral administration, transfusions are left for the infant with a hemoglobin level of about 4 gm per 100 ml or less who is debilitated from an infection, especially when signs of cardiac dysfunction are present. With such severe grades of anemia, hospitalization is safer than ambulatory treatment.

Anemia of the Premature Infant The tendency of the premature infant to develop subnormal hemoglobin levels as compared with the full term infant results from the impact of identical forces which are exaggerated in the premature infant. The differences between the anemia of prematurity and the physiologic anemia of the full term infant are quantitative. The premature infant is subject to two phases of anemia, one early and the other late. The early anemia is present at the end of the neonatal decline; the late anemia dates from that period in the first year when the iron stores are exhausted. In both the premature and full term infant the initial period is one of declining hemoglobin levels due to the decreased rate of hemoglobin synthesis. Both subsequently undergo a stage of hemoglobin regeneration from re-utilization of iron released from the destroyed red cells. The late anemia in the premature infant is one of iron deficiency when exogenous iron is not in adequate supply to meet the demands of growth. Characteristic in the premature infant are the more accelerated drop and the lower levels of hemoglobin which are obtained in the postnatal period as compared with the steadier and more prolonged drop in the hemoglobin level in the full term infant. In the premature infant, erythropoietic activity begins, therefore, at 5 to 7 weeks of age, which is earlier by a month or more than in the full term infant.

The relatively lower initial hemoglobin mass at birth of the premature infant

The total dosage according to age has also been estimated by Wallerstein and Hoag,[4] as follows: 100 mg. for infants under 6 months of age, 200 mg. under 12 months of age, 300 mg. from 12 to 24 months of age, and 400 mg. over 24 months of age. Injections are given daily or, when this is impractical, at weekly intervals. In small infants the initial dosage is 50 mg. (1 ml.). With daily injections newly formed red cells well filled with hemoglobin are recognizable in blood smears within forty-eight hours after therapy. About the fifth day of treatment reticulocyte counts reach maximum values, which are probably initiated after twenty-four hours of therapy. In patients with severe anemia the hemoglobin level reaches 11 gm. per 100 ml. after three weeks and higher in patients with moderate anemia. The magnitude of the hemoglobin response varies therefore with the degree of anemia. If hemoglobin values do not rise at least 2 gm. in three weeks, other possible causes of anemia should be considered and more iron should not be administered.

Injections into the upper outer quadrant of the gluteus muscle are given through skin which has been displaced laterally prior to the injection to prevent superficial staining. In rare cases local or generalized reactions, angioneurotic edema and recurrent arthralgia occur.[8]

RESPONSE TO ORAL IRON THERAPY. The recovery is reflected by a reticulocyte response that reaches its maximum on the fifth to the tenth day after the institution of iron therapy. The magnitude of the response is related to the degree of anemia. Following the reticulocyte rise, the hemoglobin level, volume of packed cells (hematocrit) and red cell count increase. Following the peak of the reticulocyte rise, the hemoglobin rises at an average of 0.17 gm. to 0.25 gm. per 100 ml. per day. A substantial hemoglobin rise should be observed at approximately three weeks after beginning iron therapy. The failure to achieve a level of at least 11 gm. per 100 ml. in this period with adequate iron therapy indicates that the diagnosis of anemia on a purely nutritional basis is to be questioned and suggests the continuation of an infectious process and underlying renal abnormality, a continued blood loss or impaired absorption.

It is unwise to increase the dosage in refractory cases, for it is theoretically possible to induce hemosiderosis by indiscriminate administration of excessive amounts of parenteral iron. Iron given parenterally is stored, and a maximum daily excretion of only 1 mg. compels selectivity of patients and control of treatment by this route.

ADJUVANTS TO IRON THERAPY. Adjuvants to iron are unnecessary in the treatment of iron deficiency anemia. It is unnecessary to add copper, molybdenum or cobalt supplements to obtain an adequate response. Nor are vitamin B_1 and folic acid required in treatment of iron deficiency anemia. Since iron is absorbed in the ferrous form, the reduction of ferric salts to the bivalent form depends upon reducing mechanisms present in the small intestine. Moore and his associates[43] demonstrated that the ingestion of vitamin C with ferric salts resulted in an increase in serum iron, probably by its reducing action. On the other hand, in a study of children of school age Schulze and Morgan, employing soluble ferric pyrophosphate and small supplements of copper, found that the addition of ascorbic acid was unnecessary for the synthesis of hemoglobin.

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The relatively lower initial hemoglobin mass at birth of the premature infant

the decreased synthesis of hemoglobin in the first weeks of life and the very rapid growth and expansion of blood volume constitute the fundamental factors involved in the pathogenesis of the anemia. A decreased life span in the erythrocytes of the premature infant has also been cited as a cause of the more exaggerated drop of the hemoglobin in the initial phase,[3, 4] but more extensive data are needed with regard to this factor.

Management. The greater tendency of iron deficiency in the premature infant prompts a consideration of the most favorable time for iron supplementation. Most premature infants exhaust iron stores (derived from the hemoglobin mass at birth) by the fourth month of life.[7] Seip and Halvorsen[7] found little or no stainable iron in the bone marrow of the premature infant immediately after birth and a marked increase between 4 and 8 weeks of age. Iron stores decreased until finally no hemosiderosis could be demonstrated at 6 to 12 weeks of age. In the full term infant, on the contrary, the bone marrow did not become hemosiderin free until 16 to 20 weeks of age. It is questionable whether it is necessary to administer iron prophylactically to premature infants during the postnatal period of declining hemoglobin and red cell values when the bone marrow is nonreactive and when iron from degraded hemoglobin is being conserved. Available evidence indicates that iron administered soon after birth fails to retard the postnatal drop in hemoglobin but does reveal its effectiveness in infants with higher values from the third to the fourth month. Essentially the same results are observed when iron is given by injection. When iron dextran (Imferon) was given intramuscularly over a period of two to four days during the second or third week of life, a significant increase in hemoglobin was not observed until the infant was 3 months of age. The iron administered by this method accelerates the recovery of the early anemia and prevents the late (iron deficiency) anemia.[7a]

A dosage of 30 to 45 mg. of elemental iron given from two months should protect the majority of premature infants from iron deficiency anemia and allow for iron storage. In instances in which iron deficiency already exists, the dosage is increased to 60 to 90 mg. daily. The iron is given in divided doses according to the principles outlined for iron deficiency anemia in the full term infant. In most premature infants iron should be continued for several months after the hemoglobin concentration has returned to normal to ensure adequate stores. Periodic hemoglobin determinations are required to assure the maintenance of normal blood values. Prophylaxis by means of parenteral iron (iron dextran) may be advisable before discharge from the hospital when it is anticipated that oral administration will be unreliable. The dosage in this case is 100 mg. of parenteral iron.

The management outlined for the premature infant also applies in cases of multiple births. In instances in which the hemoglobin level drops to 7 gm. per 100 ml. or less between 3 and 6 weeks of age, transfusions are required, especially when the infant is listless, eats poorly, and is not gaining weight. It should be mentioned, however, that besides the known complications of transfusions there is the possibility that repeated administration of blood may suppress inherent hematopoiesis at a time when the bone marrow may have become responsive

Iron Transport—Serum Iron and Iron Binding Capacity Recent studies dealing with the metabolism of iron with respect to the phases of absorption transport and storage have provided another diagnostic tool in characterizing an anemia based on iron deficiency Iron entering the plasma is derived from heme pigments from body stores and from gastrointestinal absorption It is transported in the plasma in combination with a specific protein a beta$_1$ globulin variously designated as metal combining globulin siderophilin or transferrin whose movement is responsible for the redistribution of iron in the body This protein carries two iron atoms in the ferric state In the process of iron turnover the transferrin delivers the metal to the liver spleen or bone marrow either for storage in a labile iron pool or for hemoglobin synthesis and erythropoiesis Iron is stored both as ferritin and as hemosiderin which are equally available for use in erythropoiesis Ferritin is soluble and not visible in the tissues whereas hemosiderin (a polymer of ferritin) is insoluble and is easily discerned as golden granules when unstained or as blue granules after staining with potassium ferrocyanide The major portion of iron leaving the plasma is normally directed toward the bone marrow where it is used for hemoglobin synthesis[3]

Normally transferrin is about one third saturated the bound fraction representing the segment linked to iron as serum iron The unsaturated or latent capacity of this protein to combine with additional iron is subject to measurement Serum iron is defined as the acid soluble iron in the serum the latent (unsaturated) iron binding capacity is defined as that amount of additional iron capable of being bound by the unsaturated protein in serum The total iron binding capacity represents the sum of these two components The percentage of saturation represents the percentage of iron binding protein which is saturated with iron and is calculated by dividing the serum iron by the total iron binding capacity The total iron binding capacity constitutes in effect an indirect estimate of the circulating iron binding protein

Granick[illegible] has suggested that iron is absorbed by the mucosal cells of the duodenum in the ferrous form and stored there as ferritin Upon entering the mucosal cells the iron apparently is oxidized to the ferric form and as ferric hydroxide polymerizes and is attached in micelles or clusters to the colorless iron free protein apoferritin It is this iron containing form which is known as ferritin A mucosal block against excessive absorption is said to occur when the available apoferritin is fully saturated with iron In the presence of an anemia it has been postulated that the relative tissue anoxemia creates a greater reducing tendency in the intestinal mucosal cells The conversion of the ferric iron present as a ferritin to circulating ferrous iron is thereby enhanced This ferrous iron then rapidly attaches itself to the iron binding protein transferrin where it is converted to the ferric form An additional amount of apoferritin is thus made available and iron is once again absorbed through the intestinal mucosa

According to this concept control of iron absorption is mediated through the intestinal mucosa which can refuse or accept iron depending upon the state of the stores Evidence has been increasingly available that in patients with refractory anemia, pernicious anemia in relapse hemolytic anemia and hemochromatosis the mucosal block does not prevent iron from being absorbed in spite of adequate body iron stores [illegible] Such data throw doubt upon ferritin and the mucosal cell as sole regulators of iron absorption and demonstrate that the mucosal block to iron absorption is only relatively complete

Except for local factors within the intestine (phosphates, phytates) the state of iron stores and the rate of erythropoiesis are major factors in the control of iron absorption.[4] Absorption is increased when the erythroid marrow is hyperactive (hemorrhage, hemolytic anemia) or when iron stores are depleted (iron deficiency). Contrariwise, absorption of iron is reduced when iron stores are increased and when erythropoiesis is depressed as in the plethora produced by transfusion.

Iron Binding Capacity of Plasma in Various Clinical Conditions. The level of serum iron reflects the balance between the iron absorbed, the iron utilized for hemoglobin synthesis, the iron released by red cell destruction and the size of the storage depots. More simply stated, at any particular time the serum iron concentration represents a precise equilibrium between the iron entering and leaving the circulation.

In persons with iron deficiency anemia the depleted stores are represented by a greatly reduced serum iron, a markedly expanded iron binding capacity and a very low percentage of saturation. An increase in the total iron binding capacity may be interpreted as an added facility for transporting this metal and is therefore an indication for iron therapy to replenish stores. In patients with acute and chronic infection the impairment of hemoglobin synthesis is accompanied by a disturbance in erythropoiesis and a diversion of iron to tissue stores.[51, 9] Both the serum iron and the latent iron binding capacity are significantly reduced but the percentage of saturation is not decreased to the extent noted in patients with iron deficiency anemia. In patients with disorders in which hemoglobin synthesis and marrow function are depressed as in those with untreated pernicious anemia, hypoplastic anemia and aplastic anemia the serum iron is greatly elevated and the latent iron binding capacity is reduced and often absent when multiple transfusions have been given. In persons with conditions of iron excess such as the hemolytic anemias, transfusion hemosiderosis and hemochromatosis the body iron is markedly increased as a result of the preponderance of red cell destruction over formation and in part from increased iron absorption. When the serum iron is markedly elevated the latent iron binding capacity is absent so that the saturation is 100 per cent. As shown in Fig. 7 and Table 11 the examination of the individual patterns materially advances the differentiation of the various anemias and provides an insight into the separate phases of iron metabolism.

Fig. 7 is based on the following average values for serum iron and latent iron binding capacity, respectively, in micrograms per 100 ml.: for normal children, 122 and 246 micrograms; iron deficiency, 27 and 472 micrograms; Cooley's anemia and the hemolytic anemias, 203 and 0 micrograms. The column for chronic infection represents a typical case in a child, in this instance with findings of 37 and 200 micrograms per 100 ml., respectively, and corresponds to the average adult figures given by Cartwright and Wintrobe[6] of 30 and 165 micrograms per 100 ml.

The values for serum iron and iron binding capacity in normal infants and children at various ages are given in Table 12.[61] The iron values noted in the period from 5 to 8 days of age represent a persistence of the findings in cord

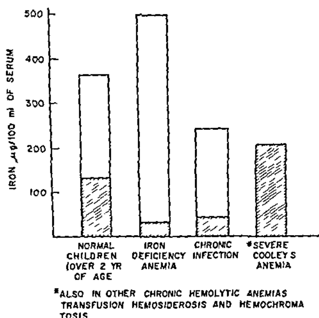


Fig 7 Schematic representation in specific anemias Shaded areas represent serum iron and clear areas latent iron binding capacity The total iron binding capacity is the sum of these two fractions The high serum iron and absent latent iron binding capacity in patients with the hemolytic anemias are contrasted with the low serum iron and greatly expanded latent iron binding capacity in those with iron-deficiency anemia

Table 11 Serum Iron and Iron Binding Capacity in Various Clinical Conditions

Decreased	*Increased*
	Serum Iron
Iron deficiency	Hemolytic diseases
Acute and chronic infection	Hemosiderosis (transfusion)
Pregnancy	Hemochromatosis
Debilitating diseases (uremia cancer etc)	Pernicious anemia (untreated)
	Cirrhosis
	Acute hepatitis
	Latent Iron Binding Capacity
Infection	Iron deficiency
Hemolytic disease	Pregnancy
Debilitating diseases	
Cirrhosis	
Hepatitis	
Pernicious anemia (untreated)	
Hemochromatosis } Absent	
Transfusion hemosiderosis } Absent	

blood—namely, a higher serum iron and lower latent and total iron binding capacities as compared to normal serum iron and higher latent and total iron binding capacities in the mother. These findings represent the combined effects of transplacental iron transfer to build up fetal stores and an increased demand for iron in the mother so that she becomes relatively iron deficient. At about

*Table 12. Normal Values of Serum Iron and Iron Binding Capacity**
(Values in Terms of Micrograms per 100 ml. Serum)

	Serum Iron	*Latent Iron Binding Capacity*	*Total Iron Binding Capacity*	*Percentage Saturation*
Adults	100	200	300	34
Infants				
5-8 days	148	114	262	65
1-2 yr.	95	319	414	22
Children				
2-6 yr.	116	279	395	28
6-12 yr.	127	213	340	38

*From Smith, C. H.: Anemias in Infancy and Childhood: Diagnostic and Therapeutic Considerations. Bull. New York Acad. Med. 30:155, 1954.

the end of the first month erythropoietic function is gradually resumed and the movement of stored iron in the direction of the bone marrow results in a low serum iron and high latent and total iron binding capacities most marked at 2 years of age. This pattern, consistent with an iron deficiency state despite the absence of anemia, indicates a continuous drain on storage depots.[67] As has been indicated, overt anemia, in which the serum iron shrinks further and the latent iron binding capacity expands, occurs with rapid growth, dietary deficiency, and other causative factors. In general there is a direct correlation between the concentrations of serum iron and the state of iron storage.

Serum iron and iron binding determinations may be employed as useful guides in gauging the adequacy and completeness of therapy in patients with iron deficiency states. Iron administered orally or parenterally in patients with iron deficiency anemia is utilized quantitatively for hemoglobin synthesis. If iron is administered parenterally only in amounts calculated to restore blood values to normal or if oral therapy is terminated as soon as normal levels are reached, the iron stores remain unreplenished. It is for this reason that iron therapy is continued for one or two months beyond the period when normal hemoglobin levels are achieved.

Hemosiderosis and Hemochromatosis. Hemosiderosis refers to excessive iron shortage in various tissues of the body without associated tissue damage. Hemochromatosis is the term applied not only to a widespread increase in storage iron but also to resultant tissue damage.[16] The clinical and pathologic changes associated with excessive dietary intake of iron among Bantu natives suffering

from malnutrition (cytosiderosis) [0] and with the deposition of iron due to multiple blood transfusions (transfusion hemosiderosis) may not always be distinguishable from those of classical hemochromatosis (bronze diabetes pigment cirrhosis) The latter a disease predominantly of middle aged men is characterized by cirrhosis of the liver diabetes skin pigmentation hypogonadism and congestive heart failure The underlying defect in this disease is a disturbance in the normal mechanism of the intestinal mucosa for preventing unnecesasry iron absorption so that excessive quantities of iron are absorbed regardless of saturated body stores [7] During the initial phase of excessive iron absorption the plasma iron is elevated and the liver and reticuloendothelial tissues show an increased iron content Eventually the liver becomes the chief site of iron storage [16]

In patients with refractory anemias such as aplastic anemia aregenerative anemia and chronic hemolytic anemia iron stores are increased by virtue of multiple transfusions (250 mg in each 500 ml of blood) greater iron absorption and prolonged iron therapy [7] Nutritional siderosis of the African Bantu natives and siderosis resulting from excessive parenteral iron therapy fall into the same category so that the complete histopathologic changes similar to classical hemochromatosis eventually develop [1]

Whether or not hemosiderosis progresses to classical hemochromatosis is still being debated Although the extensive deposition of iron in tissues is a significant characteristic of hemochromatosis this factor alone may not be the sole pathogenic factor in producing tissue changes [3] In patients with Cooley s anemia for instance chronic anemia and tissue hypoxia may constitute the essential factors in the conversion of hemosiderosis to hemochromatosis [14,30] Finch and Finch[16] state that there are no unique features in idiopathic hemochromatosis by which it can be distinguished pathologically from the terminal stage of other iron storage diseases such as dietary or transfusion hemochromatosis

Diagnostic Procedures Excessive body iron stores are indicated by an elevation of the plasma iron binding protein and excessive hemosiderin deposits in the bone marrow Actual diagnosis is only made by the demonstration of pigmentary cirrhosis of the liver

Treatment Mobilization of iron stores in idiopathic hemochromatosis has been successfully achieved by repeated phlebotomies [11,16,4] In children having huge iron stores with refractory anemia such as Cooley s anemia and pure red cell anemia the need for maintaining adequate hemoglobin levels precludes the use of this measure The intravenous injection of chelating agents increases the urinary excretion of iron but thus far has been impractical in treatment of conditions in which massive iron overload exists

Transient Dysproteinemia (Copper Deficiency in Infants) Several groups of infants have been described[30,70,7,3,84] who show pallor edema irritability associated with varying grades of hypochromic microcytic anemia hypoproteinemia hypocupremia and hypoferremia in the first year of life Hypocupremia in these infants is contrasted with the usual increase in serum copper in those with iron deficiency anemia In one group these features were attributed to a primary abnormality in plasma protein marked by an increased rate of plasma protein degradation[73] without a compensatory rate of plasma protein synthesis In other

groups patients were receiving cow's milk as their sole source of food intake.[39, 60] It is probable that two clinical states with differing causes operate in these cases,[84] one with a primary plasma protein deficiency and the other with a nutritional deficiency due to an exclusive milk diet. Improved diet, the corrective effect of copper sulfate and iron in various infants suggest that recovery occurs spontaneously and is unrelated to iron or copper therapy.[84]

Reviewing this syndrome, Schubert and Lahey[8] observed that iron deficient infants cannot only be classified into those with and those without hypocupremia and hypoproteinemia, but that intermediate or transitional forms exist. Hypoalbuminemia or hypocupremia and hypoalbuminemia occurred in infants with varying degrees of iron deficiency. The primary defect that they emphasized was one of iron deficiency, and with gradual development of moderately severe anemia a protein deficient state develops which in turn leads to dysproteinemia and copper deficiency.

Hypoproteinemia has also been shown to result not only from excessive rates of protein destruction (hypercatabolic hypoproteinemia) or from a failure of protein synthesis but also from the loss of plasma protein into the gastrointestinal tract. This process, termed exudative enteropathy,[18] can be tested by the intravenous injection of I^{131} labeled polyvinylpyrrolidone—a plasma protein substitute—which, gaining access to the lumen of the gastrointestinal tract, can be measured quantitatively by its radioactivity when excreted in the stool. I^{131} labeled polyvinylpyrrolidone is excreted in the stools of normal subjects to the extent of 0 to 1.5 per cent of the dose and up to 20 per cent in those with exudative enteropathy. Lesions of the gastrointestinal tract may or may not exist. Hypoproteinemia, edema and iron deficiency anemia have been described in children with this syndrome. Anemia in these patients in spite of the absence of gastrointestinal bleeding is explained on the basis of nonhemoglobin iron lost in the stool.[71b]

Acute Iron Intoxication. The accidental ingestion of large doses of medicinal iron preparations, usually ferrous sulfate tablets, results in a fairly characteristic set of clinical features[11, 17, 71] with a mortality of 50 per cent.[9] Early symptoms include vomiting, diarrhea, dehydration and the local effects due to the corrosive *action of the metal on the stomach and other areas of the intestinal tract.* Later evidences of severe and often irreversible cardiovascular collapse, shock and coma may be associated with the marked increases in plasma iron. Postmortem examination reveals dilatation of the right heart, pulmonary congestion, hemorrhagic and necrotizing gastritis, cloudy swelling and areas of hemorrhage in the lungs, brain, kidneys and liver.

Treatment of iron poisoning is largely symptomatic, consisting of forced vomiting and prolonged and copious gastric lavage with sodium bicarbonate solution.[9] Shock is treated with blood, plasma and oxygen. BAL and chelating agents have also been employed. Sisson and Bronson[9] have advocated gastric lavage with 20 per cent disodium phosphate dihydrate and intravenous administration of calcium disodium versenate. Blood volume and blood pressure are supported with solutions of glucose, saline or appropriate electrolytes.

Iron Deficiency Anemia in Patients With Cyanotic Congenital Heart Disease In most infants and children with cyanotic congenital heart disease the bone marrow response to the persistent anoxic stimulus is polycythemia in which the hemoglobin increase approaches the rise in the erythrocyte count Occasionally in patients the rise in hemoglobin does not keep pace with the erythrocytosis Hemoglobin levels of 10 to 13 gm per 100 ml erythrocyte counts of 6 to 8 million and hematocrit levels of 40 per cent accompanied by complaints of irritability anorexia and poor weight gain have been observed in young patients [44] Even hemoglobin levels of 16 to 18 gm per 100 ml may represent relative anemia in the presence of polycythemic blood levels The administration of iron results in marked improvement in the clinical and hematologic status Iron therapy is stopped or decreased when the hematocrit level reaches 75 per cent Experience has shown that patients with appreciable arterial unsaturation function best with a hematocrit of 55 to 75 per cent Patients with values lower than these require iron medication [44]

REFERENCES

1 Aldrich R A Acute Iron Toxicity In Wallerstein R O and Mettier S R Iron in Clinical Medicine Los Angeles 1958 University of California Press
2 Alper T and Savage D V Radioiron Studies in a Case of Hemochromatosis J Lab & Clin Med 37 665 1951
3 Bothwell T H Hurtado A V Donohue D M and Finch C A Erythrokinetics the Plasma Iron Turnover as a Measure of Erythropoiesis Blood 12 409 1957
4 Bothwell T H Pirzio Biroli G and Finch C A Iron Absorption Factors Influencing Absorption J Lab & Clin Med 51 24 1958
4a. Britton, H A Canby J P and Kohler C M Iron Deficiency Anemia Producing Evidence of Marrow Hyperplasia in the Calvarium Pediatrics 25 621 1960
5 Callender S T Iron Absorption Brit M Bull 15 5 1959
6 Cartwright G E and Wintrobe M M The Anemia of Infection a Review In Dock, W and Snapper I (editors) Advances in Internal Medicine vol. 5 Chicago 1952, Year Book Publishers Inc p 165
7 Chodos R B Ross J F Apt L Pollycove M and Halkett J A E The Absorption of Radioiron Labelled Foods and Iron Salts in Normal and Iron Deficient Subjects and in Idiopathic Hemochromatosis J Clin Invest 36 314, 1957
8 Chown B The Fetus Can Bleed Am J Obst & Gynec 70 1298 1955
9 Clark, W M Jr Jurow S S Walford R L and Warthen, R O Ferrous Sulfate Poisoning A M.A Am J Dis Child 88 220 1954
10 Coleman D H Stevens A R Jr and Finch C A The Treatment of Iron Deficiency Anemia, Blood 10 567 1955
11 Crosby W H Treatment of Haemochromatosis by Energetic Phlebotomy One Patient s Response to the Letting of 55 Litres of Blood in 11 Minutes Brit J Haemat. 4 82 1958
12 Curtis C D and Kosinski A A Fatal Case of Iron Intoxication in a Child J.A M.A 156 1326 1954
13 Dubach R Callender S T E and Moore C V Studies in Iron Transportation and Metabolism Absorption of Radioactive Iron in Patients With Fever and With Anemias of Varied Etiology Blood 3 526 1948
14 Ellis J T Schulman I and Smith C H Generalized Siderosis With Fibrosis of Liver and Pancreas in Cooley s (Mediterranean) Anemia, Am J Path 30 287 1954
14a Eng L L Chronic Iron Deficiency Anemia With Bone Changes Resembling Cooley s Anemia, Acta haemat 19 263 1958
15 Erslev A J Humoral Regulation of Red Cell Production Blood 8 349 1953

16 Finch S C and Finch C A Idiopathic Hemochromatosis an Iron Storage Disease Iron Metabolism in Hemochromatosis Medicine 34 381 1955

17 Forbes C Poisoning With a Preparation of Iron Copper and Manganese Brit M J 1 367 1947

18 *Gairdner D Marks J and Roscoe J D Blood Formation in Infancy Normal Erythropoiesis* Arch Dis Childhood 27 214 1952

19 Gardner F H The Use of Cobaltous Chloride in the Anemia Associated With Chronic Renal Disease J Lab & Clin Med 41 56 1953

20 Gillman J and Gillman T Structure of the Liver in Pellagra Arch Path 40 239 1945

21 Gillman T Lamont N Mc E Hathorn M and Canham P A S Haemochromatosis African Nutritional Siderosis and Experimental Siderosis in Animal Lancet 2 173 1957

22 Goldwasser E Jackson L O Fried W and Plzak L F Studies on Erythropoiesis the Effect of Cobalt on the Production of Erythropoietin Blood 13 55 1958

22a Gordon R S Jr Exudative Enteropathy Abnormal Permeability of the Gastrointestinal Tract Demonstrable With Labelled Polyvinylpyrrolidone Lancet 1 325 1959

23 Granick S The Chemistry and Functioning of the Mammalian Erythrocyte Blood 4 404 1949

24 Granick S Iron Metabolism and Hemochromatosis Bull New York Acad Med 25 403 1949

25 Greenwalt T J and Ayers V E Calcium Disodium EDTA in Transfusion Hemosiderosis Am J Clin Path 25 266 1955

26 Guest G M and Brown E W Erythrocytes and Hemoglobin of Blood in Infancy and Childhood Factors in Variability Statistical Studies A M A J Dis Child 93 486 1957

27 Hahn P F Carothers E L Darby W J Martin M Sheppard C W Cannon R O Beam A S Densen P M Peterson J C and McClellan G S Iron Metabolism in Human Pregnancy as Studied With the Radioactive Isotope Fe^{59} Am J Obst & Gynec 61 477 1951

27a Hammond D and Murphy A The Influence of Exogenous Iron on Formation of Hemoglobin in the Premature Infant Pediatrics 25 362 1960

28 Hegsted D M Finch C A and Kinney T D The Influence of Diet on Iron Absorption the Interrelation of Iron and Phosphorus J Exper Med 90 147 1949

29 Hollingsworth J W Life Span of Fetal Erythrocytes J Lab & Clin Med 45 469 1955

30 Howell J and Wyatt J P Development of Pigmentary Cirrhosis in Cooley s Anemia A M A *Arch Path* 55 423 1953

30a Hughes A A Kelly V J and Stewart R A The Copper Content of Infant Foods Pediatrics 25 477 1960

31 Hutchison H E The Significance of Stainable Iron in Sternal Marrow Secretions Its Application in the Control of Iron Therapy Blood 8 236 1953

32 *Josephs H W Iron Metabolism and the Hypochromic Anemia of Infancy Medicine* 32 125 1953

33 Josephs H W Absorption of Iron as a Problem in Human Physiology Blood 13 1 1958

34 Kaplan E Zuelzer W W and Mouriquand C The Significance of Visible Iron in Red Precursors A M A Am J Dis Child 86 462 1953

35 Kleckner M S Jr Kark R W Baker L A Chapman A Z and Kaplan E *Clinical* Features Pathology and Therapy of Hemochromatosis J A M A 157 1471 1955

36 Klingberg W G Jones B Allen W M and Dempsey E Placental Parabiotic Circulation of Single Ovum Human Twins A M A Am J Dis Child 90 519 1955

37 Kriss J P Carnes W H and Gross R T Hypothyroidism and Thyroid Hyperplasia in Patients Treated With Cobalt J A M A *157 117 1955*

38 Kunkel H G and Wallenius G New Hemoglobin in Normal Adult Blood Science 122 288 1955

38a Lahey M E Gubler C J Cartwright G E and Wintrobe M M Studies on Copper Metabolism VI Blood Copper in Normal Human Subjects J Clin Invest 32 322 1953

39 Lahey M E and Schubert W K New Deficiency Syndrome Occurring in Infancy A M A J Dis Child 93 31 1957

40 Langley F A Haemopoiesis and Siderosis in Foetus and Newborn Arch Dis Childhood 26 64 1951

41 Moore C V The Importance of Nutritional Factors in the Pathogenesis of Iron Deficiency Anemia Am J Clin Nutrition 3 3 1955

42 Moore C V and Dubach R Metabolism and Requirements of Iron in the Human J A M A 162 197 1956

43 *Moore C V Arrowsmith W R Welch J and Minnich V Studies in Iron Tran porta* tion and Metabolism Observations on the Absorption of Iron From the Gastrointestinal Tract J Clin Invest 18 553 1939

44 Nadas A S Pediatric Cardiology Philadelphia 1957 W B Saunders Co

45 Myerson R M and Carroll I N Treatment of Hemochromatosis by Massive Venesec tion Report of a Case Treated by Removal of Forty Liters of Blood in Twenty Eight Months A M A Arch Int Med 95 349 1955

46 Niccum W L Jackson R L and Stearns G Use of Ferric and Ferrous Iron in the Prevention of Hypochromic Anemia in Infant A M A Am J Dis Child 86 553 1953

47 Panel in Therapy The Use of Cobalt and Cobalt Iron Preparations in the Therapy of Anemia Blood 10 852 1955

48 Pommerenke W T Hahn P F Bale W F and Balfour W M Transmission of Radio-active Iron to the Human Fetus Am J Physiol 137 164 1942

49 Pribilla W Bothwell T H and Finch C A Iron Transport to the Fetus in Man In Wallerstein R O and Mettier S A Iron in Clinical Medicine Los Angeles 1958 University of California Press

50 Report of Committee on Nutrition On the Feeding of Solid Foods to Infants Pediatrics 21 685 1958

51 Robscheit Robbins F S and Whipple G H Infection and Intoxication Their Influence on Hemoglobin Production in Experimental Animal J Exper Med 63 767 1936

52 Rudolph A M Nadas A S and Borges W H Hematologic Adjustments to Cyanotic Congenital Heart Disease Pediatrics 11 454 1953

52a Schubert W K and Lahey M E Copper and Protein Depletion Complicating Hypo ferric Anemia of Infancy Pediatrics 24 710 1959

53 Schulman I Studies on the Anemia of Prematurity In Wallerstein R O and Mettier S A Iron in Clinical Medicine Los Angeles 1958 University of California Press

54 Schulman I Smith C H and Stern G S Studies on the Anemia of Prematurity A M A *Am J Dis Child 88 567 1954*

55 Schulz J and Smith N J A Quantitative Study of the Absorption of Food Iron in Infants and Children A M A J Dis Child 95 109 1958

56 Schulz J and Smith N J Quantitative Study of the Absorption of Iron Salts in Infants and Children A M A J Dis Child 95 120 1958

57 Schulze H V and Morgan A F Relation of Ascorbic Acid to Effectiveness of Iron Therapy in Children Am J Dis Child 71 593 1946

57a Seip M and Halvorsen S Erythrocyte Production and Iron Stores in Premature Infants During the First Months of Life the Anemia of Prematurity—Etiology Pathogenesis Iron Requirement Acta paediat 45 600 1956

58 Shafer A W and Marlow A A Toxic Reaction to Intramuscular Injection of Iron New England J Med 260 180 1959

58a Shahidi N T and Diamond L K Skull Changes in Infants With Chronic Iron Deficiency Anemia New England J Med 262 137 1960

59 Sisson T R C and Bronson W R Studies in the Treatment of Acute Iron Poisoning A M A J Dis Child 96 463 1958

60 Sisson T R C and Lund C J Influence of Maternal Iron Deficiency on the Newborn Am J Clin Nutrition 6 376 1958

61 Smith C H Schulman I and Morgenthau J E Iron Metabolism in Infants and

16 Finch S C and Finch C A Idiopathic Hemochromatosis an Iron Storage Disease Iron Metabolism in Hemochromatosis Medicine 34 381 1955
17 Forbes C Poisoning With a Preparation of Iron Copper and Manganese Brit M J 1:367 1947
18 Gardner D Marks J and Roscoe J D Blood Formation in Infancy Normal Erythropoiesis Arch Dis Childhood 27 214 1952
19 Gardner F H The Use of Cobaltous Chloride in the Anemia Associated With Chronic Renal Disease J Lab & Clin Med 41 56 1953
20 Gillman J and Gillman T Structure of the Liver in Pellagra Arch Path 40 239 1945
21 Gillman T Lamont N Mc I Hathorn M and Canham P A S Haemochromatosis African Nutritional Siderosis and Experimental Siderosis in Animals Lancet 2 173 1957
22 Goldwasser F Jackson L O Fried W and Plzak L F Studies on Erythropoiesis the Effect of Cobalt on the Production of Erythropoietin Blood 13 55 1958
22a Gordon R S Jr Exudative Enteropathy Abnormal Permeability of the Gastrointestinal Tract Demonstrable With Labelled Polyvinylpyrrolidone Lancet 1 325 1959
23 Granick S The Chemistry and Functioning of the Mammalian Erythrocyte Blood 4 404 1949
24 Granick S Iron Metabolism and Hemochromatosis Bull New York Acad Med 25 403 1949
25 Greenwalt T J and Ayers V F Calcium Disodium EDTA in Transfusion Hemosiderosis Am J Clin Path 25 266 1955
26 Guest G M and Brown E W Erythrocytes and Hemoglobin of Blood in Infancy and Childhood Factors in Variability Statistical Studies A M A J Dis Child 93 486 1957
27 Hahn P F Carothers E L Darby W J Martin M Sheppard C W Cannon R O Beam A S Densen P M Peterson J C and McClellan G S Iron Metabolism in Human Pregnancy as Studied With the Radio active Isotope Fe^{59} Am J Obst & Gynec 61 477 1951
27a Hammond D and Murphy A The Influence of Exogenous Iron on Formation of Hemoglobin in the Premature Infant Pediatrics 25 362 1960
28 Hegsted D M Finch C A and Kinney T D The Influence of Diet on Iron Absorption the Interrelation of Iron and Phosphorus J Exper Med 90 147 1949
29 Hollingsworth J W Life Span of Fetal Erythrocytes J Lab & Clin Med 45 469 1955
30 Howell J and Wyatt J P Development of Pigmentary Cirrhosis in Cooley's Anemia A M A Arch Path 55 423 1953
30a Hughes A A Kelly V J and Stewart R A The Copper Content of Infant Foods Pediatrics 25 477 1960
31 Hutchinson H E The Significance of Stainable Iron in Sternal Marrow Secretions Its Application in the Control of Iron Therapy Blood 8 236 1953
32 Josephs H W Iron Metabolism and the Hypochromic Anemia of Infancy Medicine 32 125 1953
33 Josephs H W Absorption of Iron as a Problem in Human Physiology Blood 13 1 1958
34 Kaplan E Zuelzer W W and Mouriquand C The Significance of Visible Iron in Red Precursors A M A Am J Dis Child 86 462 1953
35 Kleckner M S Jr Kark R W Baker L A Chapman A Z and Kaplan E Clinical Features Pathology and Therapy of Hemochromatosis J A M A 157 1471 1955
36 Klingberg W G Jones B Allen W M and Dempsey E Placental Parabiotic Circulation of Single Ovum Human Twins A M A Am J Dis Child 90 519 1955
37 Kriss J P Carnes W H and Gross R T Hypothyroidism and Thyroid Hyperplasia in Patients Treated With Cobalt J A M A 157 117 1955
38 Kunkel H G and Wallenius G New Hemoglobin in Normal Adult Blood, Science 122 288 1955
38a Lahey M E Gubler C J Cartwright G E and Wintrobe M M Studies on Copper Metabolism VI Blood Copper in Normal Human Subjects J Clin Invest 32 322 1953

CHAPTER 13

Megaloblastic Anemia and Related Anemias

Megaloblastic anemias are characterized by a predominance of megaloblasts in the marrow as well as evidence of macrocytosis in the peripheral blood and are due to a deficiency of vitamin B_{12} or folic acid. These two substances function as coenzymes in the synthesis of nucleoproteins and in their absence specific clinical and hematologic abnormalities develop.

Vitamin B_{12}. Vitamin B_{12} is found principally in foods of animal origin such as liver, kidney and muscle and to a lesser extent in eggs and milk. It is not usually present in higher plants but can be synthesized by certain bacteria, among them *Streptomyces griseus*. It occurs also as a by-product of streptomycin. In its pure form vitamin B_{12} is a crystalline substance containing cobalt whose chemical name is cyanocobalamin. It functions as "extrinsic factor" in regard to erythropoiesis and in the presence of the "intrinsic factor" of Castle secreted by gastric mucosa it is absorbed into the small intestine and passes into the blood stream to the liver, where it is stored for use by the bone marrow and other tissues. The erythrocyte maturing factor (EMF) is probably identical with the extrinsic vitamin B_{12} after its absorption. Vitamin B_{12} exists in the serum mainly in combined form as a heat labile complex with serum protein, specifically in the alpha globulin fractions.[6] Normal serum has a limited capacity to bind crystalline vitamin B_{12} in vitro.

Folic Acid. Folic acid, also a member of the vitamin B complex, is distributed in plant and animal tissues, chiefly in fresh leafy green vegetables such as spinach and cabbage and in liver, kidney, yeast and mushrooms. Liver, beef and fresh green vegetables are the common foods with a high folic acid content. Folic acid is readily absorbed from the gastrointestinal canal. In its synthesized form it is known as pteroylglutamic acid (PGA). For its metabolically active form it must be converted from its conjugated state to folinic acid (citrovorum factor) or to tetrahydrofolic acid.[14]

Formiminoglutamic acid (FIGLU), a normal intermediate in amino acid metabolism, requires the active tetrahydrofolic acid for its further degradation to glutamic acid. When tetrahydrofolic acid is insufficient, formiminoglutamic acid accumulates and its excretion in the urine thus serves as a useful indicator of clinical folic acid deficiency.[18,33] Metabolic correction of the deficiency following administration of folic acid can be shown by the elimination of the increased urinary formiminoglutamic acid.

Children Serum Iron and Iron Binding Protein Diagnostic and Therapeutic Implications In Levine S Z (editor) Advances in Pediatrics vol 5 Chicago 1952 Year Book Publishers Inc p 195

62 Smith N J Iron as a Therapeutic Agent in Pediatric Practice J Pediat 53 37 1958

63 Smith N J Considerations of Iron Metabolism in Early Infancy J Pediat 54 654 1959

64 Stevens A R Jr Coleman D H and Finch C A Iron Metabolism Clinical Evaluation of Iron Stores Ann Int Med 38 199 1953

65 Strauss M B Anemia of Infancy From Maternal Iron Deficiency in Pregnancy J Clin Invest 12 345 1933

66 Sturgeon P Studies of Iron Requirement in Infants and Children the Influence on Normal Infants of Oral Iron in Therapeutic Doses Pediatrics 17 341 1956

67 Sturgeon P Iron Metabolism a Review Pediatrics 18 267 1956

68 Sturgeon P Studies of Iron Requirements in Infants and Children In Wallerstein R O and Mettier S A Iron in Clinical Medicine Los Angeles 1958 University of California Press

69 Sturgeon P Studies of Iron Requirements in Infants Influence of Supplemental Iron During Normal Pregnancy on Mother and Infant the Infant Brit J Haemat 5 45 1959

70 Sturgeon P and Brubaker C Copper Deficiency in Infants a Syndrome Characterized by *Hypocupremia Iron Deficiency Anemia and Hypoproteinemia* A M A J Dis Child 92 254 1956

71 Thompson J Two Cases of Ferrous Sulfate Poisoning Brit M J 1 640 1947

71a Ulstrom R A Exudative Enteropathy Pediatrics 24 521 1959

71b Ulstrom R A and Krivit W Exudative Enteropathy Hypoproteinemia Edema and Iron Deficiency Anemia Thirtieth Annual Meeting Society of Pediatric Research Paper No 27 Swampscott Mass May 3 1960

72 Ulstrom R A Smith N J and Heimlich E M Transient Dysproteinemia in Infants a New Syndrome Clinical Studies A M A J Dis Child 92 219 1956

73 Ulstrom R A Smith N J Nakamura K and Heimlich E Transient Dysproteinemia in Infants Studies on Protein Metabolism Using Amino Acid Isotopes A M A J Dis Child 93 536 1957

74 Wallerstein R O and Hoag M S Treatment With Iron Dextran of Iron Deficiency Anemia in Children J A M A 164 962 1957

75 Wallerstein R O and Robbins S L Hemochromatosis After Prolonged Oral Therapy in a Patient With Chronic Hemolytic Anemia Am J Med 14 256 1953

76 Whipple G H Sisson T R C and Lund C J Delayed Ligation of the Umbilical Cord Its Influence on the Blood Volume of the Newborn Am J Obst & Gynec 10 603 1957

77 Wickster G Z and Christian J R Posthemorrhagic Shock in the Newborn Premature Pediat Clin North America 1 555 1954

78 Widdowson E M and Spray C M Chemical Development in Utero Arch Dis Childhood 26 205 1951

79 Wintrobe M M Factors and Mechanisms in the Production of Red Corpuscles Harvey Lect 45 87 1949 1950

80 Wintrobe M M Clinical Hematology ed 4 Philadelphia 1956 Lea & Febiger

80a Wintrobe M M Cartwright G E and Gubler C J Studies on the Function and Metabolism of Copper J Nutrition 50 395 1953

81 Woodruff C W *Maternal Factors in Hypochromic Anemia in Infancy* J A M A 162 659 1956

82 Woodruff C W Multiple Causes of Iron Deficiency in Infants J A M A 167 715 1958

83 Woodruff C W and Bridgeforth E P Relationship Between the Hemogram of the Infant and That of the Mother During Pregnancy Pediatrics 12 681 1953

84 Zipursky A Dempsey H Markowitz H Cartwright G and Wintrobe M M Studies on Copper Metabolism Hypocupremia in Infancy *A M A J Dis Child* 96 148 1958

ogenesis of this disease The occasional case in which a response is obtained with large doses of vitamin B_{12} is explained by the sparing action on folic acid[18] Vitamin B_{12} not only interacts with folic acid in the tissues as a coenzyme at different stages of nucleic acid synthesis but also influences in some way the storage and intake of folic acid[23] A disorder identical with that in the human being has been produced in monkeys fed a diet largely of powdered milk notorious for its causative relationship to the disease in infants Without additional ascorbic acid the animals develop scurvy and then megaloblastic anemia[19] On the other hand the severe deficiency of folic acid as a complication of scurvy in monkeys fed milk diets is probably due to nonspecific factors operating in scurvy[1]

The participation of ascorbic acid in pathogenesis is emphasized by the fact that the incidence of megaloblastic anemia has dropped precipitously since infant foods have been fortified with large amounts of this vitamin This dramatic change applied particularly to powdered milk preparations which had been low in ascorbic acid There is reason to believe that many cases reported as goat's milk anemia are indeed megaloblastic anemia of infancy

Clinical Features The onset is insidious and usually makes its appearance between the ages of 2 and 17 months with a mean age of 7 to 8 months[19] Pallor anorexia irritability failure to gain weight persistent upper respiratory infections intermittent fever and diarrhea are common Petechiae mucous membrane hemorrhages and ecchymoses in conjunction with thrombocytopenia are present in severely affected infants The nutritional status is poor The heart may be enlarged and some degree of hepatomegaly is a common feature Splenomegaly is unusual There is no neurologic component as in patients with pernicious anemia There may be transitory gastric achlorhydria even after histamine administration and occasional hypoproteinemia

Laboratory Findings Anemia is mild or severe Hemoglobin levels below 5 gm per 100 ml are common[4] The red blood cell count may be markedly reduced in occasional cases to less than 1 million per cubic millimeter The blood smear shows anisocytosis poikilocytosis and either a normochromic or hypochromic normocytic anemia Occasional or moderate macrocytosis is noted Except for leukocytosis with infections leukopenia and neutropenia are common findings The polymorphonuclear neutrophils are enlarged and hypersegmented The platelets are reduced in number often markedly so In moderately or severely affected infants nucleated red cells appear in the peripheral blood The bone marrow examination is diagnostic The megaloblastic type of erythropoiesis corresponds to that of pernicious anemia The open structure of the nucleus its finely divided chromatin easily visible parachromatin and basophilic cytoplasm characterize the megaloblast[3, 4] Giant metamyelocytes and giant stab cells with distorted nuclei are pathognomonic of this disorder Appearing at times in advance of megaloblasts they are indications of the early stages of megaloblastic anemia The variation in the number of megaloblasts normoblasts and abnormal metamyelocytes in different cases indicate that the deficiency may be partial and not always complete Formiminoglutamic acid urinary excretion has been found useful as an indicator of folic acid deficiency[18]

Treatment The anemia responds readily and completely to oral or parenteral

Folic acid deficiency results principally from inadequate dietary intake, increased demand, antagonists to its utilization (such as occur in antileukemia therapy), and interference with intestinal absorption. Occasionally, ascorbic acid deficiency contributes to the causation of megaloblastic anemia by limiting the conversion of folic acid to its active form.

Interrelationship of Folic Acid and Vitamin B_{12}. Folic acid is closely associated with vitamin B_{12} in the synthesis of nucleic acids, acting as catalysts at different stages of this process.[3] There is evidence also that a balance exists between folic acid and vitamin B_{12} and that a deficiency of one increases the requirement for the other.[7,40] Whereas a deficiency of either substance results in megaloblastic bone marrow and glossitis, the nervous system disturbance of pernicious anemia, for instance, is caused solely by vitamin B_{12} deficiency. The recovery from neurologic symptoms requires vitamin B_{12} and not folic acid therapy. The deficiency of folic acid or vitamin B_{12} not only results in the appearance of megaloblasts but also affects other cells in the bone marrow. The constituents of nucleic acids (uracil, thymine, and thymidine) possess therapeutic activity in patients with megaloblastic anemia but not to the extent that folic acid and vitamin B_{12} do.[3]

The following megaloblastic anemias are due to the absence of gastric intrinsic factor or result from dietary deficiencies of either folic acid or vitamin B_{12}. Treatment consists of giving one or both of these factors, depending upon the extent of the response.

1. Megaloblastic anemia of infancy
2. Juvenile pernicious anemia
3. Nutritional macrocytic anemia
4. Impaired absorption from the gastrointestinal tract
   - A. Sprue (tropical), idiopathic steatorrhea (nontropical sprue), celiac disease
   - B. Congenital strictures, gastrointestinal resections, anastomoses
5. Miscellaneous
   - A. Infestation with fish tapeworm
   - B. Anticonvulsant drugs
     - (1) Phenytoin sodium (Dilantin)
     - (2) Primidone (Mysoline)
   - C. Chronic liver disease
   - D. Acute leukemia during treatment with antimetabolite drugs
   - E. Sickle cell anemia and other hemolytic anemias
   - F. Hemochromatosis

MEGALOBLASTIC ANEMIA OF INFANCY

Etiology. Megaloblastic anemia of infancy is an acute anemia (rarely chronic), transitory in nature, due to a deficiency of folic acid. It is brought on by states in which reserves of folic acid are greatly diminished, such as during periods of rapid growth, high nutritional demands, infection, and prolonged diarrhea.[9,46] Premature infants are especially susceptible. It is of interest that all types of milk used for infant feeding provide a low intake of folic acid, and goat's milk is especially poor in vitamin B_{12}, yet few infants develop the disease. Ascorbic acid and, to some extent, vitamin B_{12} appear to play significant roles in the path

minished or absent gastric secretion of intrinsic factor Histamine fast achlorhydria and a characteristic type of gastric atrophy[4, 13] accompany the defective gastric secretion Continuous treatment is required to maintain the patient in a state of remission Without vitamin B_{12} the erythroblast fails to mature and assumes the cytologic features of the megaloblast The absorption of substances other than vitamin B_{12} is usually normal A familial tendency toward the disease has been noted[8, 34]

In its complete form juvenile pernicious anemia is characterized as in the adult by a macrocytic anemia megaloblastic bone marrow atrophy of the papilla of the tongue and other features of recurrent glossitis nervous system involvement a specific response to parenteral injections of vitamin B_{12} or liver extract converting the bone marrow to a normoblastic pattern and the need for continuous therapy to prevent relapses Histamine refractory achlorhydria one of the cardinal features of adult pernicious anemia is an inconstant finding however in the juvenile form of the disease

Clinical Manifestations Many of the cases are detected during the first two years of life whereas others become manifest during childhood until puberty The onset is insidious and pallor apathy fatigability anorexia and diarrhea are early symptoms A beefy red sore tongue papillary atrophy and other features of recurrent glossitis are common Signs of subacute dorsolateral degeneration of the spinal cord occur infrequently[5] Most common are neurologic signs including ataxia less often paresthesia of the hands and feet impaired vibratory perception Babinski signs and absence of tendon reflexes Jaundice is absent and there is no enlargement of the liver and spleen

Laboratory Findings Macrocytes many of them oval anisocytosis poikilocytosis polychromasia and occasionally nucleated red cells characterize the blood smear and parallel the severity of the anemia The hemoglobin ranges from 4 to 9 gm per 100 ml usually below 6 gm The red blood cell count is comparatively lower than the hemoglobin level varying from 1 to 2 5 million per cubic millimeter

The anemia being macrocytic shows a high color index and an increase in the mean corpuscular volume (MCV) ranging from 110 to 140 cubic microns which is well above the upper limit of normal of 92 cubic microns The life span of the red cells is moderately shortened[8] The macrocyte normally saturated with hemoglobin takes a dark stain and conveys the impression of hyperchromia Supersaturation in nonexistent Leukopenia is common and may be marked with the number of white cells ranging from 3 000 to 4 000 per cubic millimeter and is caused by a depression of myeloid tissue in the bone marrow Hypersegmentation of oversized polymorphonuclear leukocytes (macropolycytes) with bizarre shaped lobes is common The platelets are reduced in number The leukopenia and thrombocytopenia are not so marked as to lead to susceptibility to infection or purpura

The bone marrow is hyperplastic with the nucleated red cells ranging up to 50 per cent of the total cells Of these the megaloblasts in different stages of maturation and hemoglobinization predominate Mitotic figures are common The abnormally large size of the polymorphonuclear cells and the metamyelocytes

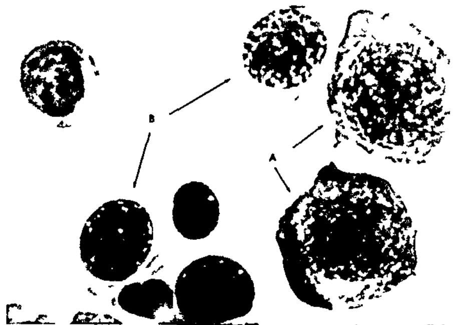


Fig 8 Bone marrow smears from a patient with megaloblastic anemia of infancy showing megaloblastic erythroid precursors (×900) Note the more typical megaloblastic chromatin structure in the early erythroblasts *A* and the less well defined changes in the normoblasts *B* Cell at upper left is a small lymphocyte (From Lubby L Megaloblastic Anemia in Infancy *J Pediat 54 617 1959*)

administration of folic acid given daily in a dosage of 10 to 30 mg over a period of two to three weeks or until the blood and bone marrow picture returns to normal A reticulocyte rise follows the administration of folic acid Complete disappearance of the megaloblastic pattern is noted two days after the injection of folic acid[4] Liver extract is of value but it is not so direct or reliable as folic acid Ascorbic acid should be given simultaneously in a dose of approximately 200 mg if there is any suspicion of its deficiency Some workers advocate a combination of folic acid and ascorbic acid from the outset as a routine procedure In countries where malnutrition and poverty exist and megaloblastic anemia is prevalent vitamin B_1 is also added[1] An enriched diet containing folic acid and vitamin B_1 is essential Antibiotic therapy is important especially since infection plays such an important role in etiology Transfusions are indicated when anemia is severe but folic acid must be given at the same time

Prognosis Complete recovery within a few weeks is expected following folic acid therapy Although the administration of folic acid produces a permanent cure of megaloblastic anemia of infancy juvenile pernicious anemia with a similar megaloblastic bone marrow is a lifelong disease requiring continuous therapy

JUVENILE PERNICIOUS ANEMIA

Etiology Pernicious anemia in childhood is exceedingly rare [9] Addison ian pernicious anemia is a megaloblastic anemia caused by a deficiency of vitamin B_1 which is based on a defective absorption of the vitamin resulting from di

minished or absent gastric secretion of intrinsic factor Histamine fast achlorhydria and a characteristic type of gastric atrophy[4, 43] accompany the defective gastric secretion Continuous treatment is required to maintain the patient in a state of remission Without vitamin B_1 the erythroblast fails to mature and assumes the cytologic features of the megaloblast The absorption of substances other than vitamin B_1 is usually normal [illegible] A familial tendency toward the disease has been noted [8, 34]

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are characteristic features The changes are particularly pronounced among the metamyelocytes which are giant in size possess a U shaped nucleus and have a bulky poorly granulated cytoplasm

Diagnosis Because of the rarity of pernicious anemia in children it is important to eliminate other causes of megaloblastic anemia The difficulty in diagnosis has been the presence of free hydrochloric acid *in the gastric contents* and the inconsistency of histamine refractory achlorhydria in many of the reported cases [9] Long term follow up is necessary to determine whether achlorhydria essential for the diagnosis of the adult disease will eventually develop The presence of a megaloblastic bone marrow the absence of intrinsic factor a good reticulocyte response following the administration of vitamin B_{12} and the continuous requirement of vitamin B_{12} to maintain a remission of the disease constitute the basic diagnostic features

A young man who was reported to have had pernicious anemia at the age of 13 months was found to be still suffering from vitamin B deficiency at 18 years of age [28] He secreted normal amounts of acid and pepsin in the gastric juice but was unable to absorb a test dose of radioactive vitamin B unless this was given with a source of intrinsic factor The gastric juice was deficient in intrinsic factor but the gastric mucosal membrane was histologically normal The patient s father suffered from typical addisonian pernicious anemia It was suggested that vitamin B_{12} deficiency itself could cause temporary gastric atrophy which becomes permanent and complete if treatment is withheld too long In another study[15] two children with pernicious anemia showed normal gastric function and structure An inability to absorb vitamin B from the gastrointestinal tract was demonstrated The author postulated that the gastric manifestations of pernicious anemia are not an intrinsic part of the disease but are secondary to chronic deficiency of vitamin B

A number of tests have been devised for the confirmation of the diagnosis of pernicious anemia [10] The newer methods include biopsy of the gastric mucosa[4, 47] to determine the presence of atrophic changes estimation of serum vitamin B_{12} level and methods for determining the amount of radioactive vitamin B_{12} absorbed from the intestine The urinary excretion method of Schilling [31] one of the simplest consists of the oral administration of a small dose of radioactive vitamin B_{12} followed by a flushing dose of 1 mg of nonradioactive vitamin B_{12} injected parenterally immediately or within two hours Following the oral dose of radioactive vitamin B_{12} substantial amounts of the vitamin are excreted in the urine of normal persons within twenty four hours as compared with minute amounts in patients with pernicious anemia In patients with pernicious anemia serum vitamin B_{12} levels are low

Course and Prognosis With modern treatment utilizing vitamin B_{12} in adequate dosage the outlook for reversal of the disorder is excellent Infections and complications arising from nervous system involvement interfere with treatment

Treatment The response to liver extract and vitamin B_{12} is rapid and specific The standard form of treatment consists of the parenteral injection of vitamin B_{12} The dosage is to some degree an individual matter In children a remission of the disease should be obtained with doses of 15 to 30 micrograms given every day or two for two to four weeks followed by a maintenance dose of the same amount given every two to four weeks Large doses may be required An alternate schedule is to give a large initial dose daily for 5 injections or every second

or third day for ten to fifteen days followed by 1 injection of 100 micrograms each month[9] Liver extract may be given on the basis of its vitamin B_{12} content

The reticulocytes begin to increase on the third to fourth day rise to a maximum on the sixth to eighth day and fall gradually to normal about the twentieth day[9] The height of the reticulocyte count is inversely proportional to the degree of anemia Beginning bone marrow reversal from megaloblastic to normoblastic cells is obvious within six hours and is completely normoblastic in seventy two hours

Prompt hematologic responses are also obtained with the use of oral folic acid Folic acid has the disadvantage of having no effect upon neurologic manifestations and has been known to precipitate or accelerate their development Iron is occasionally required when a generally inadequate diet has been given which is deficient in this mineral

NUTRITIONAL MEGALOBLASTIC ANEMIA

Nutritional megaloblastic anemia is a macrocytic anemia occurring among underprivileged peoples in tropical and subtropical countries where malnutrition is prevalent due to poverty and dietary habits[41] It results from an inadequate dietary intake of folic acid and vitamin B_{12} The megaloblastic bone marrow macrocytic anemia and leukocytic changes respond to folic acid with or without vitamin B_{12} [1] Correction of dietary defects is essential Iron and transfusions are given as needed

This type of anemia is to be differentiated from that occurring in kwashiorkor The latter originally described as a nutritional syndrome of African infants and children is also prevalent in Central and South America[3] India China and other countries and results from severe protein deficiency The intake of calories derived largely from starchy food is adequate The anemia is usually normochromic and normocytic and less commonly macrocytic and microcytic[1] Occasionally it is aggravated by blood loss due to hookworm and malaria The bone marrow is normoblastic or megaloblastic[1,3] The anemia of kwashiorkor has also been found in association with aplastic crises (erythroblastopenia) in which giant proerythroblasts appeared in the marrow[16] Patients with the normochromic normocytic type of anemia respond to a well balanced diet rich in protein but not in liver extract folic acid or vitamin B_{12} Iron is occasionally effective In those cases of kwashiorkor in which megaloblastic anemia of infancy is diagnosed the bone marrow reverts to normal following the administration of folic acid[39]

Nutritional megaloblastic anemia of pregnancy is also more common in underprivileged areas and is due to a chronic dietary deficiency The prognosis for the mother and fetus has improved with the use of folic acid and vitamin B_{12} [41]

MISCELLANEOUS MEGALOBLASTIC ANEMIAS

Megaloblastic Anemia With Hemolytic Anemias

Megaloblastic anemia is a rare complication of hemolytic anemia and has been reported in association with acquired hemolytic anemia hereditary spherocytosis

thalassemia sickle cell anemia and paroxysmal nocturnal hemoglobinuria The *megaloblastic bone marrow is attributed to folic acid deficiency caused by the* demands for this substance imposed by rapidly developing erythroid cells The need for folic acid exceeds the dietary intake and results in tissue depletion

Megaloblastic Anemia With Hemochromatosis

The occurrence of a macrocytic anemia with a megaloblastic type of erythropoiesis has been observed in hemochromatosis Its causation as well as its formation and absorption is related to the associated pigmentary cirrhosis hindering the storage of antianemia factors In some patients liver therapy has been effective[1] in one patient there was a striking response to folic acid but not to vitamin B_{12} [13]

Megaloblastic Anemia With Fish Tapeworm

The fish tapeworm *(Diphyllobothrium latum)* leads to megaloblastic anemia indistinguishable from pernicious anemia This type of anemia is caused by competition of the tapeworm with the host for the vitamin B_{12} in the food in the intestinal tract necessary for the nutrition of the parasite[39] Treatment depends upon expulsion of the parasite or the administration of liver or vitamin B_{12}

Megaloblastic Anemia Caused by Anticonvulsant Therapy

Extremely rare causes of megaloblastic anemia are the anticonvulsant agents phenytoin sodium (Dilantin)[30] and primidone (Mysoline) [11] The corrective effect of folic acid suggests that phenytoin and primidone may act as competitive inhibitors of some enzyme system normally involving folic acid[11] A conditioning factor for the development of anticonvulsant megaloblastic anemia may be the presence in the patient of occult nutritional deficiency secondary to an inadequate diet particularly of folic acid and to some extent of vitamin C

MALABSORPTION SYNDROMES—SPRUE (TROPICAL) IDIOPATHIC STEATORRHEA (NONTROPICAL SPRUE) AND CELIAC DISEASE

Except for the common factor of impaired motility and absorption of the small intestine there are no specific anatomic or pathologic changes in patients with sprue idiopathic steatorrhea or celiac disease These diseases show a marked *variation with regard to clinical features and causation*[10] *Although the passage* of stools containing an excess of fats (steatorrhea) is a characteristic feature of all of these diseases the fecal content of other nutrients is also increased Thus the body loses vitamins minerals proteins carbohydrate and such hematopoietic substances as folic acid vitamin B_{12} and iron Diarrhea weakness and wasting are prominent symptoms

Tropical sprue has a seasonal incidence and is limited largely to certain overcrowded and underdeveloped geographic areas in tropical and subtropical climates It develops abruptly and runs a rapid course Whether it is primarily or only secondarily a deficiency disease is not known [4]

Idiopathic steatorrhea (nontropical sprue) is less common occurs sporadically has no seasonal or geographic incidence and is usually a chronic and insidious disease [6]

Celiac disease is included by some authors with nontropical sprue since it may persist into adult life Celiac disease (Gee Herter disease) a chronic disorder of infants and children is characterized by diarrhea wasting (especially noticeable in the flattened buttocks and groins) retardation of growth and abdominal distention The proteins (glutens) of wheat and rye are precipitating factors of the disorder the gliadin fractions acting as toxic rather than allergic agents [37]

In most cases of tropical sprue a macrocytic anemia indistinguishable from addisonian pernicious anemia is present and is associated with a megaloblastic bone marrow Although a macrocytic anemia is commonly found in patients with nontropical sprue it only occasionally presents the characteristics of pernicious anemia and may not be associated with megaloblastic bone marrow [6] Glossitis is present in patients with tropical and nontropical sprue Folic acid vitamin B_{12} and liver extract are specific therapeutic agents in the treatment of patients with tropical sprue with megaloblastic anemia The deficiency pattern including diarrhea takes longer to reverse Folic acid vitamin B_{12} and liver extract are also helpful in patients with nontropical sprue with megaloblastic anemia although hematologic remission is not always complete and there is no demonstrable effect on the steatorrhea Hypochromic microcytic anemia is by far more common than the macrocytic type in celiac disease Iron administered orally or parenterally is given for the iron deficiency and folic acid liver extract and vitamin B_{12} are given for the macrocytic anemia

The megaloblastic anemia associated with intestinal strictures fistulas and anastomoses is in some way dependent upon the invasion of the small intestine by organisms normally inhabiting the colon In fistulas such as the ileocolic variety there is a direct contamination by organisms from the colon In strictures and blind loops stagnation predisposes to abnormal bacterial growth and absorption of toxic substances [4,36] Most of the clinical and hematologic features are those of pernicious anemia or tropical sprue Vitamin B_{12} and especially folic acid have a beneficial effect on the anemia Surgical correction of the intestinal disorder is often followed by relief of the anemia but long term parenteral vitamin B_{12} *administration may still be necessary When intestinal stasis is a factor in the* course of intestinal stricture and anastomosis the impaired absorption of vitamin B_{12} may be significantly improved by administration of the tetracycline group of antibiotics [14]

NORMOBLASTIC MACROCYTIC ANEMIAS

The normoblastic macrocytic anemias constitute a heterogenous group in which the bone marrow is normoblastic but macrocytes appear in variable numbers in the peripheral blood interspersed among the red cells of a normocytic normochromic anemia These macrocytic anemias are secondary to aplastic anemia lymphomas and primary nonhematopoietic diseases such as hypothyroidism scurvy and liver disease

REFERENCES

1 Adams I B Anemia in Kwashiorkor Brit M J 1 537 1951
2 Anderson I M Frazer A C French J M Hawkins C I Ross C A C and Simmons H C The Influence of Gluten and Antibacterial Agents on Fat Absorption in the Sprue Syndrome Gastroenterology 81:98 1951
3 Benjamin B Infantile Form of Pernicious (Addisonian) Anemia Am J Dis Child 75 143 1948
4 Cameron D G Watson C M and Witts L J The Clinical Association of Macrocytic Anemia With Intestinal Stricture and Anastomosis Blood 4 793 1949
5 Chanarin I Dacie J V and Mollin D L Folic Acid Deficiency in Haemolytic Anaemia Brit M J 5 245 1959
6 Comfort M W and Wallaeger L I Nontropical Sprue Pathology Diagnosis and Therapy A M A Arch Int Med 98 807 1956
7 Cooke W T Frazer A C Peeney A L P Sammons H C and Thompson M D Anomalies of Intestinal Absorption of Fat the Haematology of Idiopathic Steatorrhea Quart J Med 17:9 1948
8 Dacie J V The Haemolytic Anaemias Congenital and Acquired New York 1954 Grune & Stratton Inc
9 deGruchy G C Clinical Haematology in Medical Practice Springfield Ill 1958 Charles C Thomas Publisher
9a Flexner J M and Hartmann R C Megaloblastic Anemia Associated With Anticonvulsant Drugs Am J Med 28 386 1960
10 Girdwood R H The Megaloblastic Anaemias Their Investigation and Classification Quart J Med 25 87 1956
11 Girdwood R H and Lenman J A R Megaloblastic Anaemia Occurring During Primidone Therapy Brit M J 1 146 1956
12 Gomez F Galvan R R Cravioto J and Silvester F Malnutrition in Infancy and Childhood With Special References to Kwashiorkor In Levine S Z (editor) Advances in Pediatrics Chicago 1955 Year Book Publishers Inc
13 Granville N and Dameshek W Hemochromatosis With Megaloblastic Anemia Responding to Folic Acid New England J Med 258 586 1958
14 Greenberg G R and Jaenicke L On the Activation of the One Carbon Unit for the Biosynthesis of Purine Nucleotides In Ciba Foundation Symposium The Chemistry and Biology of Purines Boston 1957 Little Brown & Co p 204
14a Halsted J A Lewis P M and Gasster M Absorption of Radioactive Vitamin B_{12} in the Syndrome of Megaloblastic Anemia Associated With Intestinal Stricture or Anastomosis Am J Med 20 42 1956
15 Koszewski B J Occurrence of Megaloblastic Erythropoiesis in Patients With Hemochromatosis *Blood 7 1182 1952*
15a Laskin S L Pernicious Anemia in Childhood Pediatrics 25 91 1960
16 Lien Keng K Erythroblastopenia With Giant Pro erythroblasts in Kwashiorkor Blood 12 171 1957
17 Lien Keng K and Oding O Megaloblastic Anemia in Infancy and Childhood in Djakarta A M A J Dis Child 97 209 1959
18 Luhby A L Megaloblastic Anemia in Infancy Clinical Considerations and Analysis J Pediat 54 617 1959
19 May C D Nelson E N Lowe C V and Salmon R J Pathogenesis of Megaloblastic Anemia in Infancy Am J Dis Child 80 191 1950
20 May C D Stewart C T Hamilton A and Salmon R J Infection as a Cause of Folic Acid Deficiency and Megaloblastic Anemia A M A Am J Dis Child 84 718 1952
21 May C D Hamilton A and Stewart C T Experimental Megaloblastic Anemia and Scurvy in the Monkey Nature of the Relation of Ascorbic Acid Deficiency to the Metabolism of Folic Acid Compounds J Nutrition 49 121 1953

22 Mollin D L Baker S J and Doniach I Addisonian Pernicious Anemia Without Gastric Atrophy in a Young Man Brit J Haemat 1 278 1955
23 Nieweg H O Faber J C de Vries J A Stenfert Kroese W F *Relationship of Vitamin B and Folic Acid in Megaloblastic Anemias J Lab & Clin Med 44 118 1954*
24 Dameshek W (moderator) Panels in Therapy the Treatment of Sprue Blood 11 570 1956
25 Peterson J C and Dunn S C Pernicious Anemia in Childhood Am J Dis Child 71 252 1946
26 Pitney W R Beard M F and van Loon E J Observations on the Bound Form of Vitamin B in Human Serum J Biol Chem 207 143 1954
27 Reisner E H Jr The Nature and Significance of Megaloblastic Blood Formation Blood 13 313 1958
28 Reisner E H Jr Wolff J A McKay R J Jr and Doyle E F Juvenile Pernicious Anemia Pediatrics 8 88 1951
29 Rosensweig L and Bruton O C Pernicious Anemia in an 8 Year Old Girl Pediatrics 6 269 1950
30 Ryan G M S and Forshaw J W B Megaloblastic Anaemia Due to Phenytoin Sodium Brit M J 2 242 1955
31 Schilling R F Intrinsic Factor Studies the Effect of Gastric Juice on the Urinary *Excretion of Radioactivity After the Oral Administration of Radioactive Vitamin* B_{12} J Lab & Clin Med 42 860 1953
32 Scrimshaw N S Behar M Perez C and Viteri F Nutritional Problems of Children in Central America and Panama Pediatrics 16 378 1955
33 *Spray G H and Witts L J Excretion of Formiminoglutamic Acid as an Index of Folic* Acid Deficiency Lancet 2 702 1959
34 Stamos H F Heredity in Pernicious Anemia Am J M Sc 200 586 1940
35 Sunberg R D Schaar F and May C D Experimental Nutritional Megaloblastic Anemia *Hematology* Blood 7 1143 1952
36 Thompson R B and Ungley C C Megaloblastic Anemia Associated With Anatomic Lesions in the Small Intestine Blood 10 771 1955
37 van de Kamer J H Weijer H A and Dicke W K Coeliac Disease an Investigation Into the Injurious Action on Patients With Coeliac Disease Acta paediat 42 223 1953
38 Von Bonsdorff B and Gordin R Antianemic Activity of Dried Fish Tapeworm Acta med scandinav (suppl 266) p 283 1952
39 Walt F *Holman S and Hendrickse R G Megaloblastic Anemia of Infancy in* Kwashiorkor and Other Diseases Brit M J 1 1199 1956
40 Welch A D and Heinle R W Hematopoietic Agents in Macrocytic Anemias Pharmacol Rev 3 345 1951
41 Wills L Pernicious Anemia Nutritional Macrocytic Anemia and Tropical Sprue a Discussion Blood 3 36 1948
42 Wood I J Value of Gastric Biopsy in the Study of Chronic Gastritis and Pernicious Anemia Brit M J 2 283 1951
43 Wood I J Doig R K Motteram R Weiden S and Moore A The Relationship Between the Secretions of the Gastric Mucosa and Its Morphology as Shown by Biopsy Specimens Gastroenterology 12 949 1949
44 Wood, J L Pl thora in the Newborn Infant Associated With Cyanosis and Convulsions a Review of Postnatal Erythropoiesis J Pediat 54 143 1959
45 Zuelzer W W and Ogden F H Megaloblastic Anemia of Infancy Am J Dis Child 71 211 1946
46 Zuelzer W W and Rutzky J Megaloblastic Anemia of Infancy In Levine S Z (editor) Advances in Pediatrics Chicago 1953 The Year Book Publishers Inc

CHAPTER 14

Hypoplastic and Aplastic Anemias

General Considerations The hypoplastic and aplastic anemias constitute a heterogeneous group of anemias which include several well defined entities and a larger number of still obscure hypoplastic states reflecting degrees of erythropoietic suppression. These anemias are not caused by excessive hemolysis, blood loss nor *in the majority of instances by space occupying infiltrations of the bone marrow*. The cardinal feature of this group of anemias is bone marrow hypofunction resulting from a failure of red cell production and hemoglobin synthesis to compensate for the normal rate of blood destruction. Erythropoiesis alone may be significantly decreased or the defect may also extend to the granulocytes and platelets, resulting in a peripheral blood picture of pancytopenia. Bone marrow aspiration or biopsy reveals either the anticipated aplasia or a specimen varying from normal cellularity to hypercellularity, in which case depressed functional activity is postulated.[16] The difficulties of reconciling the state of the bone marrow with the peripheral blood picture and the resistance of the hypoplastic aplastic group to all forms of antianemia therapy except transfusion have prompted their designation as refractory anemias.[3] Abnormalities in the metabolism of nucleic acids, particularly DNA, have been postulated as a cause of many refractory *anemias in which peripheral pancytopenia is accompanied by* a cellular hyperplastic bone marrow.[9a]

The anemias are usually normochromic and normocytic, occasionally macrocytic with moderate anisocytosis, and are distinguished from other blood dyscrasias by a diminished number of reticulocytes and other evidences of inactive blood formation. The absence of enlargement of lymphnodes, liver and spleen in patients with the major anemias of this group contributes a feature of diagnostic importance.

Classification For purposes of simplification these anemias have been consolidated into two general categories—the hypoplastic and aplastic anemias. Although overlapping obviously occurs, there are members of each group nevertheless which possess such well defined characteristics as to constitute specific entities.

Aplastic anemia is characterized by a simultaneous depression of the three principal cellular elements in the bone marrow and a peripheral picture of profound anemia, leukopenia, neutropenia and thrombocytopenia. It can be noted from the accompanying classification that aplastic anemia includes both primary

and secondary forms although the former are more prominent in infancy and childhood

Hypoplastic anemia differs from aplastic anemia in that the formation of red cells is impaired, with lesser or no involvement of granulocytes and platelets In recent years the term hypoplastic anemia has been applied rather loosely to intermediate or miscellaneous conditions Reports of cases of hypoplastic anemia now range from conditions in which there is a failure of red cell production to those in which leukocytes and platelets are simultaneously depressed but to a lesser degree than red cells This tendency has been exaggerated by the knowledge that a transient anemia accompanied by a diminished reticulocyte count need not necessarily be correlated with a bone marrow deficient in erythroid tissue To one of the members of this group pure red cell anemia, a restrictive connotation should be applied This is a chronic congenital aregenerative anemia which had been previously placed into a separate category[4] but which is now classified with the hypoplastic anemias in contrast to the acquired forms of erythroblastopenia

1 Hypoplastic anemia (major effect on erythropoiesis)
  A Idiopathic—hypoplasia confined to erythropoiesis
    (1) Congenital—pure red cell anemia (chronic congenital aregenerative anemia)
  B Acquired—with varying degrees of erythrocytic hypoplasia
    (1) Anemias due to infections drugs chemicals toxins autoimmune and allergic states
    (2) Aplastic crisis in course of hemolytic syndromes such as hereditary spherocytosis and sickle cell anemia
    (3) Anemia resulting from suppressive effect of multiple transfusions on erythropoiesis
    (4) Miscellaneous—moderate anemia with or without lesser involvement of granulocytes and platelets
2 Aplastic anemia (severe pancytopenia)
  A *Idiopathic or primary*
    (1) Congenital aplastic anemia
      (a) With multiple congenital anomalies (Fanconi type)
      (b) Without associated anomalies
    (2) Nonfamilial and without associated anomalies
  B Acquired—secondary
    (1) Anemia due to antimicrobial and other chemotherapeutic agents
    (2) Anemia due to industrial and household chemicals
    (3) Anemia due to irradiation
    (4) Miscellaneous causes—infections endocrine factors bone marrow invasion or replacement (myelophthisic anemias) hypersplenism

HYPOPLASTIC ANEMIAS

Idiopathic Hypoplastic Anemia—Pure Red Cell Anemia

Definition Pure red cell anemia is also called chronic congenital aregenerative anemia congenital hypoplastic anemia pure red-cell aplasia primary erythroid hypoplasia, erythrogenesis imperfecta etc The term pure red cell anemia[7] refers to a chronic progressive anemia confined to a failure of erythropoiesis without an equivalent depression of the white blood cells or platelets[8, 9] The

bone marrow is characterized by a complete or almost complete absence of cells of the erythroid series with normally proliferating cells of the granulocytic series and megakaryocytes The failure of the bone marrow to provide adequate numbers of erythrocytes results in a persistent and progressive anemia with the need for long term supportive transfusions Pure red cell anemia has been observed at all ages but it is particularly conspicuous as a congenital disease in early infancy and childhood

Pathogenesis The etiology is unknown but several theories have been advanced to explain a condition in which only a single element of the bone marrow is affected It is conceivable that the hematopoietic defect results from a noxious factor operating at critical periods of fetal development Pure red cell anemia has been described in association with other development defects such as a hypoplastic and cystic kidney [45] and complete[10] and temporary[18] pure red cell anemia has been reported in patients with autoimmune hemolytic disease Pure red cell anemia may conceivably result from a similar mechanism—namely the action of such antibodies against the red cells in fetal life producing prolonged and permanent depression of erythropoiesis [44] Pure red cell anemia may be compared with other congenital anomalies caused by developmental defects

Anthranilic acid a metabolite formed in the breakdown of tryptophan is excreted in relatively large quantities when a riboflavin deficient animal is fed tryptophan A similar defect in tryptophan metabolism has been reported in a number of patients with congenital pure red cell anemia [1] The administration of riboflavin to these patients resulted in a decrease in the excretion of anthranilic acid but did not alter the hematologic status

Clinical Features Pure red cell anemia has an insidious onset with progressive pallor anemia irritability listlessness and anorexia which are usually apparent at the age of 2 to 3 months or later in the first year The infant appears healthy at birth and general development proceeds normally Exceptionally the onset of the disease may be delayed such a delay has been reported in a child 6 years old [9] and two brothers 17 years of age [3] This occurrence in siblings has also been noted in other cases [3]

Except for pallor there is little to be observed at physical examination The liver spleen and lymph nodes are not enlarged jaundice is absent and there are no manifestations of bleeding In some patients a particular type of facies has been described [4] consisting of tow colored hair snub noses thick upper lips rather wide set eyes and an intelligent expression

The liver and spleen enlarge as transfusion hemosiderosis develops Normal red cell survival which is present when the spleen is not enlarged is shortened as this organ becomes the site of increased red cell destruction Long standing administration of blood results in increased pigmentation of the skin This is due to both iron deposition and excessive melanin formation [6] Cardiac enlargement multiple arrhythmias and refractory heart failure are frequently inevitable consequences

Pure red cell anemia also occurs as an acquired erythrocytic hypoplasia in adults without a proved common etiology [41, 3] Infection allergy red cell autoantibodies in hemolytic anemia and chemical exposure have been incriminated

A more constant finding is the coexistence of this disease with benign thymoma[5, 6, 339]

Laboratory Findings The outstanding feature is a chronic aregenerative and refractory anemia in which there is a striking absence from the marrow of the nucleated precursors of the red cells without a simultaneous depression of granulocytes platelets and their precursors The anemia is normocytic and normochromic with reticulocytes low in number or absent Without supportive transfusions the hemoglobin falls to extremely low values

The number of platelets white blood cells and differential counts are normal Marked leukopenia and moderate depression of platelets occur in rare instances in which the spleen enlarges and manifests a hypersplenic function The bone marrow in such a patient shows normal myeloid activity and a normal number of megakaryocytes In a boy 7½ years old under observation splenectomy resulted in a return of leukocytes and platelets to normal but erythropoiesis remained hypoplastic The serum iron level is elevated with complete saturation of the latent iron binding capacity as a result of prolonged transfusion therapy

In this condition also the bone marrow frequently contains varying numbers of primitive cells termed hematogones[43, 1] Although such cells usually are smaller than the ordinary lymphocytes they resemble them closely in size and morphology They possess a dense homogenous matlike nucleus and a narrow rim of nongranular basophilic cytoplasm in which lymphocytes are usually entirely absent These primordial cells possibly represent progenitors of the erythrocytic series In one patient they were replaced by small numbers of normoblasts following the administration of cortisone

Pathologic Findings The essential feature is widespread hemosiderosis at times associated with evidence of hemocromatosis In a 10 year old patient who died of heart failure postmortem examination revealed the typical syndrome of hemochromatosis including diabetes mellitus and skin pigmentation The heart was greatly enlarged and many of the myocardial fibers contained iron pigment scanty interstitial fibrosis and occasionally necrosis of isolated fibers In a 9 year old child whose terminal illness was severe hepatitis postmortem examination revealed massive liver necrosis and generalized hemosiderosis with fibrosis of the liver pancreas and thyroid The myocardial fibers were hypertrophied vacuolated and contained moderate amounts of hemosiderin

Diagnosis In a typical congenital case the disease is fully established in early infancy The need for frequent transfusions is soon found to be based on erythropoietic hypoplasia in the bone marrow Occasionally pure red cell anemia may follow erythroblastosis and be mistaken for the protracted depression of erythropoiesis which sometimes accompanies the latter disease Pure red cell anemia can be differentiated from the hemolytic anemias such as sickle cell disease thalassemia major and hereditary spherocytosis by the lack of splenomegaly and the absence of icterus reticulocytes and morphologic abnormalities in the red cell

There is as yet no unanimity as to the degree of erythroid hypoplasia which the name of this anemia implies Although the designation of pure red cell anemia relates to conditions in which the bone marrow is practically devoid of normoblasts (less than 2 per cent) in some reports these elements though

reduced are nevertheless present in moderate numbers The myeloid erythroid ratio which should be necessarily high in a typical case may be only moderately reduced in others In one report[1] the disorder is referred to as "erythrogenesis imperfecta in which several bone marrow examinations showed a variation between erythroid hypoplasia and hyperplasia These cases however share the common feature of varying aplasia of erythropoiesis without involvement of leukocytes or platelets The differentiation from aplastic anemia and the acquired erythroblastopenia is will be discussed subsequently

Treatment Major therapy consists of transfusions ACTH and steroids and splenectomy

Transfusions Supportive transfusions to maintain the hemoglobin at levels compatible with freedom from symptoms constitute a basic need The use of sedimented or packed cells is preferable to whole blood As a guide to management we found that with few exceptions patients in this category of chronic anemia do not require transfusions until hemoglobin levels drop to 7 to 7 5 gm per 100 ml at which point clinical symptoms usually appear These include anorexia listlessness apathy and incipient signs of heart failure It is apparent that hemosiderosis is to be expected following *repeated transfusions Another limitation* of frequent transfusions is their potential depressant effect upon endogenous erythropoiesis and hemoglobin synthesis [46]

ACTH and Steroids In association with transfusions adrenocortical hormones and ACTH are given primary consideration since they represent a potent form of therapy [1, 1, 33] *Depending upon the age of the patient the steroid hormones* (prednisone prednisolone and like synthetic substances) are prescribed in a daily dosage of 15 to 60 mg given in divided doses every six hours (a total of 15 to 20 mg in children under 2 years of age and up to 60 mg in older children) With optimal dosages evidences of the ability of the bone marrow to *produce red cells should be manifested within three to four weeks If and when* the patient responds the dosage should be reduced to the minimum at which a remission is maintained (approximately 15 mg daily in divided doses)

A favorable response is reflected in a bone marrow and blood remission—with erythroid hyperplasia reticulocytosis and increased hemoglobin and *red cell levels Any evidences of infection should prompt the administration* of a suitable antibiotic Despite the persistently low hemoglobin levels these patients are not unduly susceptible to infection If no response is obtained with the steroids in the prescribed period ACTH by intramuscular or subcutaneous injection should be substituted preferably as the gel in a daily dosage of 40 mg *in the older child Here too the response varies with each patient so that no fixed* amount can always be prescribed With a favorable response reducing and stopping the drug entirely should be attempted periodically to determine the capacity of the patient to establish spontaneous erythropoietic function

It has been recommended that steroids be given early in the course of treatment *since no response* is to be expected when hemosiderosis has resulted after prolonged transfusion therapy [19] In our experience only one of six patients with congenital pure red cell anemia responded favorably to steroid therapy—in a 17 month old patient In another patient of equal age in this group steroid treatment

was ineffective In the remaining four patients steroids were given at various intervals during the course without effect In another series[1] on the other hand ten patients responded favorably Intermittent corticosteroid therapy was recommended to obviate growth and bone age retardation and osteoporosis It was postulated that the mechanism of action was stimulation of an enzyme system possibly tryptophan oxidase Reticulocytosis occurred at about nine days following treatment with a peak response of 12 to 18 per cent between fourteen and twenty two days Most patients had a normal hemoglobin level within six weeks and two patients maintained this improvement without further treatment Allen and Diamond[1] caution the need for early treatment since none of the patients who had the disease for more than three years responded whereas all children who had disease for three months or less did respond

Splenectomy Splenectomy which is frequently recommended only as a last resort in the treatment of pure red cell anemia has nevertheless proved effective on occasion both in reducing the number of transfusions and in restoring erythropoiesis[10, 3] When transfusions are required at increasingly frequent intervals to maintain a physiologically sufficient although reduced hemoglobin level it is postulated that an extracorpuscular hemolytic component has developed which is presumably located in the spleen Accelerated destruction can be confirmed by tagging normal donor red cells with radioactive chromium and noting their shortened survival

In one of our patients with pure red cell anemia marked enlargement of the spleen was present This finding is contrary to an accepted criterion of the aplastic hypoplastic group of anemias—namely the failure to palpate the spleen In this patient however removal of the spleen resulted in extending the interval between transfusions although depression of erythropoiesis persisted Undoubtedly in patients without enlargement splenectomy is justified when a hemolytic factor for donor blood is demonstrated by techniques revealing a shortened survival of transfused normal erythrocytes The presence of such a hemolytic factor would be clinically suspected when regularly spaced intervals for transfusions are suddenly shortened

In patients in whom no hemolytic component is apparent splenectomy is considered on the basis that an abnormally functioning spleen may depress the erythropoietic function of bone marrow This procedure is recommended when ACTH the steroids and other therapeutic agents alone or in combination have been given extensive trial without success There is less reluctance to remove the spleen when the bone marrow is not entirely devoid of erythroid elements in any stage of development In any event the procedure may be justified on the basis of a splenic humoral factor which inhibits maturation of precursors either visualized or potentially present in unidentified primordial cells such as hematogones The fear that removal of the spleen deprives the patient of needed resources of erythropoietic tissue is not justified by results in patients with this disease Although the majority of patients are not improved by splenectomy the condition is not aggravated Occasionally splenectomy alone has a beneficial effect in restoring erythropoiesis In other patients cortisone and other therapeutic agents have proved more effective after splenectomy Increased

experience may show that splenectomy will be efficacious in patients who re spond initially to steroid therapy [9]

Prognosis In most patients the course of congenital pure red cell anemia is one of chronic and progressive anemia with a fatal outcome In selected patients supportive transfusion therapy ACTH and steroids and splenectomy can main tain a concentration of hemoglobin compatible with health Long term trans fusions however carry the hazards of hemosiderosis and hemochromatosis Re ported cases suggest that a more favorable response to steroid therapy could be expected in patients with moderate numbers of normoblasts in the bone marrow than in those in whom the normoblasts were practically depleted In one of our patients however no response with corticosteroids was obtained despite an initial finding of 5 per cent normoblasts

An important factor in evaluating therapy and in a consideration of prognosis is the possibility of spontaneous recovery Such remissions occurred in three of twelve patients cited by Diamond[7] and in others such as those reported by Palmen and Vahlquist [31] Remission can occur at any period from early childhood to adolescence with stabilization at a lower hemoglobin level

Death results from cardiac failure hepatitis during the course of transfusions and overwhelming infection and sepsis In these terminal events the factor of hemosiderosis from excessive iron deposition resulting from transfusions and hemochromatosis may conceivably play an important complicating role

Acquired Hypoplastic Anemias

Anemia Due to Infections, Drugs Chemicals Toxins, and Autoimmune and Allergic States Under the heading of acute erythroblastopenias Gasser[16] de scribed temporary iregenerative crises of one week s duration resulting in severe anemia in children as a result of toxic infectious or allergic causes This acquired type of pure red cell anemia was noted in patients who had been given drugs such as amidopyrin santonin and barbiturates in those with infections including mumps atypical pneumonia bacterial sepsis due to meningococcus and staphylo coccus and in those with autoimmune hemolytic disease [18]

Aplastic Crisis The aplastic crisis occurring in patients with a number of hemolytic conditions notably hereditary spherocytosis has been discussed else where Crises in these conditions are not hyperhemolytic as originally supposed but are aplastic The bone marrow shows a complete absence of erythropoiesis the occasional presence of proerythroblasts a shift to the left of the granu locytic series and a decrease of megakaryocytes The severity of the anemia is aggravated by the fact that the aplastic state occurs in patients with pre existing hemolytic disease in which the life span of the red cell is already reduced

Anemia Resulting From Suppressive Effect of Multiple Transfusions on Erythropoiesis The suppressive effect of multiple transfusions on erythropoiesis which has been demonstrated in connection with chronic hemolytic anemias[16] probably extends also to the refractory anemias in which transfusions are the mainstay of treatment

Miscellaneous The miscellaneous group of hypoplastic anemias is by far

the most common Persistent ill defined anemias in children are often un diagnosed or are suspected of being an intermediate stage of idiopathic aplastic anemia The normochromic and normocytic anemia which is found is at variance with the bone marrow which is normal both with respect to total cellularity and in the content of erythroid elements The anemia is moderately severe resists treatment with iron and other hematinics and may be corrected periodically by the administration of blood Not until the basic difficulty such as a specific hypersensitivity or the source of chronic upper respiratory infections for instance is eliminated or treated adequately will the hemoglobin level return to normal

APLASTIC ANEMIA (BONE MARROW FAILURE REFRACTORY ANEMIA)

Definition Aplastic anemia refers to a refractory anemia characterized by severe anemia leukopenia and thrombocytopenia and is usually associated with aplasia or hypoplasia of the bone marrow Ehrlich is credited with the first description of the disease in a young adult in 1888[9] The term aplastic anemia gained popularity as increasing numbers of cases with pancytopenia often in the absence of a hypoplastic marrow were reported

Etiology The search for a history of exposure to agents injurious to the hematopoietic system is often difficult to obtain in children so that the majority of cases of aplastic anemia in younger age groups are eventually classified as idiopathic

In a series of 334 patients with aplastic anemia Wolff[a] found 24 3 per cent under the age of 15 years Of the total number of patients 57 2 per cent gave no history of possible exposure to a toxic compound and were classified as having idiopathic anemia 23 6 per cent had been exposed to one or more antibacterials shortly before the onset of the disease 9 per cent had had an exposure to other types of possible toxic materials and 10 2 per cent gave a combined exposure history In another series Scott and co-workers[10] found that seventeen of thirty nine patients (thirteen below 20 years of age) could give no history of exposure to known or suspected marrow depressants and were therefore considered to have the idiopathic disease

Aplastic anemia is classified as either idiopathic or secondary caused by a drug chemical or ionizing radiation It is possible that patients who give no history of toxic exposure and are classified as having idiopathic anemia might well have been exposed to an unrecognized agent In some patients an interval of several weeks may elapse following exposure to a toxic agent before clinical signs and symptoms appear Even with a reliable history of exposure the relationship between the toxic agent and bone marrow depressant may have been fortuitous Idiopathic and secondary cases of aplastic anemia have similar clinical pictures except that patients with the secondary type have a somewhat better prognosis

Idiopathic Aplastic Anemia

Included among the cases of bone marrow failure of unknown etiology are two distinctive syndromes—one associated with congenital anomalies and the other without anomalies

Congenital Aplastic Anemia With Multiple Congenital Anomalies (Fanconi Type) The best known group of congenital malformations in which anemia constitutes an outstanding symptom is represented by the Fanconi syndrome. This condition includes the rare association of pancytopenia, bone marrow hypoplasia, and a number of congenital anomalies—namely, a patchy brown pigmentation of the skin due to a deposition of melanin, hypogenitalism, microcephaly, dwarfism, strabismus, ptosis of the eyelids, nystagmus, exaggerated deep tendon reflexes, mental retardation, anomalies of ears, deafness, and skeletal abnormalities. Anomalies of the thumb, radii, long bones, congenital dislocation of the hips, syndactyly, congenital heart disease, and kidney anomalies have been noted in various reports.[6] Originally, Fanconi[13] in 1927 reported cases occurring in three siblings. Sporadic as well as familial cases have been described. In most of the reported cases hematologic abnormalities are initially detected between 4 and 12 years of age. An earlier onset has been observed in one infant at 13 months of age, and in two brothers symptoms of a blood disorder appeared at the ages of 19 and 20 years, respectively.[18] The anemia is normocytic and slightly macrocytic, and leukopenia and thrombocytopenia coexist. As in patients with aplastic anemia, the bone marrow content varies from acellular to hypercellular.

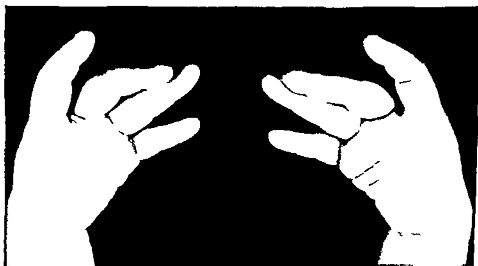


Fig. 9. Fanconi's syndrome showing bilateral absence of thumbs.

The Fanconi syndrome appears to be transmitted by an autosomal recessive gene with variable penetrance. Treatment consists of supportive transfusions, steroid hormones, antibiotic therapy for infections, and splenectomy in selected cases. The severe bleeding tendency associated with thrombocytopenia is difficult to control, and in one patient this was accomplished by splenectomy.[6] Patients with hypoplasia or maturation arrest have also been benefited by splenectomy.[34] Indications for splenectomy are the same as in other types of aplastic anemia. Death usually results from hemorrhage into the brain or gastrointestinal

tract[6] Although the outlook is extremely grave for the majority of patients there are exceptional ones whose lives have been prolonged by modern therapy or whose survival is explained by minimal depression of the bone marrow

Congenital Hypoplastic Anemia Without Associated Anomalies Estren and Dameshek[1] have reported several siblings in each of two families with generalized quantitative hypoplasia of all the elements of the bone marrow with the nucleated red cells in normal or elevated percentages In one of their patients showing severe anemia with an increased number of reticulocytes and thrombocytopenia splenectomy resulted in moderate clinical and hematologic improvement

Acquired (Secondary) Aplastic Anemias

Depressed blood formation may result from various forms of injury to the bone marrow The clinical features are identical with those of primary aplastic anemia but secondary anemia offers a better prognosis especially if the condition is diagnosed early and the offending agent is eliminated

Anemia Due to Antimicrobial and Other Chemotherapeutic Agents The offending drugs and chemicals are of two types those which regularly produce bone marrow depression when given in sufficient dosage (such as the compounds used in the treatment of leukemia and malignant lymphomas) and substances which are occasional bone marrow depressants [4] In the antileukemia group are nitrogen mustard the folic acid antagonists 6 mercaptopurine busulfan (Myleran) and triethylenemelamine Included in this category also as depressants are roentgen rays radioactive phosphorus radium and products of atomic fission or atomic bomb explosions

In the category of the occasional offenders are those drugs and chemicals which produce aplastic anemia with the initial dosage or in a subsequent course Tridione Mesantoin and chloramphenicol are among the more common therapeutic agents which depress the bone marrow Less common bone marrow depressants are sulfanilamide sulfathiozole and sulfapyridine and among the antibiotics tetracycline streptomycin and penicillin Atabrine neoarsphenamine among the organic arsenicals gold and Butazolidin are also important etiologic agents but are of lesser importance in pediatric practice

In the pathogenesis of aplastic anemia chloramphenicol was probably the most frequent cause of aplastic anemia in the United States between 1949 and 1952 [8] A sharp decrease in the total number of cases followed 1952 when the hazard was pointed out and precautions were advised [40] Because of a recent increase in the number of patients in whom chloramphenicol was associated with the development of aplastic anemia the cautious use of this drug has been re emphasized [34]

Anemia Due to Industrial and Household Chemicals Benzol benzene and benzene derivatives among the household and industrial chemicals have a high priority among the marrow depressants because of their widespread use as solvents and in the manufacture of drugs dyes shellac paint removers and lacquers Of importance also are DDT and other insecticides which are especially injurious in concentrated exposure within enclosed and poorly ventilated areas

Anemia Due to Irradiation Roentgen rays and other forms of ionizing radiation are well known physical agents which cause marrow depression Radiations are of two kinds The first are those which penetrate the body tissues such as the roentgen rays (x rays) gamma rays and fast and slow neutrons Gamma rays and neutrons are emitted from the explosion of atomic bombs and reach the bloodforming tissues rapidly The second type are the alpha and beta particles which have a limited range of tissue penetration They damage bone marrow directly when introduced into the body as sometimes occurs in radium dial workers[30]

Except when given in tremendous amounts irradiation damages the precursors of the hematopoietic system rather than the formed elements in the peripheral blood The effect on the blood elements is related to their life span (the leukocytes having the shortest and the red cells the longest life span) the individual radiosensitivity of the precursors (the lymphocyte precursors being most sensitive) and the ability of hematopoietic tissue to regenerate (lymphatic tissue being most reactive) The leukocytes therefore show the first changes (namely a decrease in the number of lymphocytes being most noticeable) followed by a reduction in the number of platelets and red cells Granulocytopenia and thrombocytopenia lead to infection and hemorrhage Neutropenia is usually among the first manifestations of chronic overexposure Individual susceptibility dosage and period of exposure account for the variation in blood alterations

Miscellaneous Causes Untreated pernicious anemia overwhelming infections and mechanical interference with erythropoiesis in the bone marrow may produce a blood picture of aplastic anemia Anemias resulting from bone marrow invasion or replacement are the *myelophthisic anemias due to bone marrow encroachment* by foreign cells as occurs in patients with leukemia lymphosarcoma Hodgkin s disease Niemann Pick disease Gaucher s disease Letterer Siwe disease Albers Schonberg disease (osteopetrosis) and myelofibrosis

Features Common to Idiopathic and Acquired Types of Aplastic Anemia

Pathology The basic features of aplastic anemia are due to alterations of the bone marrow During the course of the disease the bone marrow shows a progressive decrease in the total count so that the megakaryocytes eventually disappear the myeloid elements and nucleated red cells are greatly reduced and the lymphocytes predominate in the smears Patients with normal or hyperplastic bone marrows with a peripheral blood picture of aplastic anemia have been diagnosed as having maturation arrest or bone marrow block Quantitative variations in the total nucleated cell count and distribution of the blood elements may be due to the fact that areas of relatively acellular marrow alternate irregularly with areas of normal or increased cellularity When aplastic the normally red marrow appears yellow and fatty The occasional increase in reticulocytes is due to patchy areas of active blood formation in the bone marrow The liver spleen and lymph nodes are unaltered Hemochromatosis may coexist with hemosiderosis due to iron deposition from multiple transfusions [6]

Clinical Features The onset is insidious rarely abrupt with anemia and

cutaneous hemorrhages as early features It is soon noted that the thrombocytopenia and purpura are insufficient to account for the degree of anemia The clinical features are closely dependent upon the blood changes anorexia weakness easy fatigue and dyspnea on exertion from anemia and loss of blood Epistaxis bleeding from the gums and gastrointestinal and renal tracts menorrhagia and retinal hemorrhage are prominent manifestations in later stages Neutropenia is associated with sore throat and ulcerations of the mouth and pharynx Fever due to secondary infections may be difficult to control with antibiotics The spleen liver and lymph nodes are not enlarged

Laboratory Findings The red blood cell count fluctuates between 1 5 and 3 million per cubic millimeter the hemoglobin drops proportionately The reticulocytes number below 2 per cent and are sometimes absent The red cells are normochromic and normocytic but macrocytes are often numerous The white cells tend to range between 1 500 and 4 000 per cubic millimeter principally due to the lack of granulocytes The platelets fall below 50 000 per cubic millimeter Bone marrow aspiration discloses aplasia or hypoplasia During the course of transfusion therapy the serum iron already elevated in this disease becomes markedly increased and the iron binding protein becomes saturated resulting in an absent latent iron binding capacity [4]

Differential Diagnosis True aplastic anemia with an acellular bone marrow and the typical picture of profound anemia leukopenia neutropenia and thrombocytopenia is a rare occurrence in infancy and childhood The marrow reveals progressive hypoplasia although scattered areas of normal composition may be noted in samples from different sites The anemia develops insidiously so that diagnosis is difficult before pancytopenia is fully developed

In differential diagnosis of aplastic anemia the following causes of pancytopenia are to be considered

1 Leukemia with aleukemic or subleukemic blood picture
2 Aplastic anemia
3 Bone marrow infiltration or replacement
   A Malignant lymphomas
      (1) Hodgkin s disease
      (2) Lymphosarcoma
      (3) Reticulum cell sarcoma
   B Metastatic carcinoma
   C Rare
      (1) *Osteopetrosis (marble bone disease)*
      (2) Myelofibrosis myelosclerosis
4 Hypersplenic syndromes
   A Banti s disease
   B Lymphomas
   C Gaucher s disease
   D Niemann Pick disease
   E Letterer Siwe disease
   F Splenic panhematopenia
   G Disseminated lupus erythematosus
5 Miscellaneous
   A Megaloblastic anemias
   B Overwhelming infections

Hemorrhage based on thrombocytopenia or a blood picture of chronic anemia may constitute the dominant features until the deficiencies of the other cellular elements appear As already mentioned thrombocytopenic purpura which may be considered early in the course of the disease is associated with more than moderate anemia only as a result of blood loss In the absence of overt hemorrhage the anemia must therefore be explained on another basis

Acute lymphoblastic leukemia in the leukopenic stage may simulate aplastic anemia but microscopic study of aspirated marrow usually reveals the precise nature of the illness With an acellular marrow the use of concentrated and volumetric bone marrow aspiration facilitates the differentiation between the two diseases [48] A *contracted or absent myeloid erythroid (nucleated cell) layer* and normal or increased percentage of fat in aplastic anemia are contrasted with leukopenia from other causes in which the fatty layer is absent and the myeloid erythroid layer is variable

Enlargement of the lymph nodes and spleen usually present in patients with acute leukemia is absent in those with aplastic anemia In patients with splenic panhematopenia and secondary hypersplenism the spleen is predominantly implicated in the pathogenesis of pancytopenia and the bone marrow contains its full quota of each of the cellular elements Myelofibrosis and neoplastic infiltration are also to be considered in the differential diagnosis

Management Comprehensive treatment consists of preventive measures search for and elimination of the etiologic agent transfusions and other supportive measures stimulation of hematopoiesis and a consideration of splenectomy

Preventive Measures Because hypersensitivity is so variable and occurs so unexpectedly a selection of a drug which is potentially myelotoxic requires some deliberation and an attempt at supervision should be instituted if the drug is eventually to be used These considerations apply particularly to patients with a history of allergic manifestations such as eczema urticaria chronic rhinitis hay fever or asthma or who in the past have had skin rashes or constitutional reactions following exposure to a chemical or drug

The extent to which precautionary measures are carried out depends therefore upon the known depressant effect of the drug the benefits to be derived from its use and the inherent nature of the patient with respect to hypersensitivity reactions Myelotoxic drugs are difficult to control because bone marrow hypoplasia can develop suddenly and because it is difficult to isolate the sensitive individual in whom treatment might be complicated by hematopoietic depression Even with drugs such as Tridione Mesantoin Butazolidin and chloramphenicol which are known offenders the incidence of toxicity is extremely low in comparison with the large number of patients so treated Nevertheless a hemoglobin test white blood cell count and blood smear should be made periodically on patients receiving such drugs over prolonged periods and especially when treatment is intermittent The exact intervals between these examinations cannot be specified In the case of chloramphenicol weekly counts should be done initially and even more often when suspicious signs of toxicity appear Fever petechiae purpura epistaxis oozing from the gums mouth ulcerations malaise weakness fatigue and skin rashes are important signs of bone marrow depression In

the case of chloramphenicol withdrawal of the drug at the stage of granulopenia and anemia usually results in complete hematopoietic recovery [11] [8] In the marrow the signs of existing or impending bone marrow depression consist of a sudden shift to an immature level the presence of promyelocytes and myelocytes or marked reduction in the more mature elements and an increase in stem cells and lymphocytes

Removal of the Causative Agent For obvious reasons recognition and elimination of the causative agent (such as a drug chemical insecticide or household chemical) are the first steps of treatment Removal of the patient from further contact with the suspected toxic agent should be carried out immediately

Transfusions and Other Supportive Measures Transfusions to combat anemia and provide platelets and ACTH and the adrenocortical hormones to stimulate hematopoiesis are administered as for pure red cell anemia Infections are often difficult to control even with the liberal use of wide spectrum antibiotics Reliance is placed on penicillin streptomycin and tetracycline until more specific information is obtained about the invading organism from blood cultures and examination of other accessible infected sites In the event of neutropenia antibiotics are given if there are other clinical manifestations of active disease The prophylactic use of antibiotics is hazardous because of possible bacterial resistance

Children with proved aplastic anemia have recently been treated by Shahidi and Diamond[4] with testosterone combined with corticosteroids over prolonged periods with resulting clinical and hematologic improvement During the remission the hemoglobin reached normal levels the number of reticulocytes increased and the bone marrow was converted from a markedly hypocellular and fatty marrow to a hypercellular one with erythroid hyperplasia More extensive experience is needed with this promising approach

Testosterone was used as testosterone propionate or methyltestosterone in the form of 10 mg buccal Linguets in a daily dosage of 1 or 2 mg per kilogram of body weight Corticosteroids were given in addition as triamcinolone in a dosage ranging from 8 to 12 mg daily Testosterone is also given intramuscularly for those who cannot manage the Linguets Although the dosage is still empirical the d ug has been given in doses of 100 mg once or t ice a week

Control of Hemorrhage Thrombocytopenia almost invariably leads to excessive bleeding which is difficult to control and is often responsible for a fatal outcome Transfusions of fresh whole blood and platelet rich plasma (obtained after sedimentation or centrifugation) collected in plastic or siliconized equipment supplies intact platelets or platelet material for brief periods Reliance is placed on ACTH and adrenocortical steroids to control hemostasis This is accomplished more by increase of capillary resistance than by elevation of the number of platelets [37]

Bleeding in aplastic anemia including the Fanconi type is occasionally amenable to splenectomy In two girls 9 and 12 years of age diminution of generalized bleeding and severe menorrhagia respectively resulted from splenectomy when all other available measures for controlling hemostasis failed In a 15 year old girl with the Fanconi type of aplastic anemia splenectomy was

performed because of severe thrombocytopenia and bleeding At 20 years of age she was no longer anemic and the bleeding tendency had subsided but thrombocytopenia and leukopenia persisted [6]

Marrow Transplantation Efforts to suppress or abolish the immune response to permit marrow transplantation have not as yet proved successful [53]

Splenectomy The rationale for splenectomy has been reviewed in connection with pure red cell anemia Recently this procedure has been recommended more freely in patients with unexplained refractory anemia especially when any improvement in bone marrow function could be predicted In one series six of twelve patients with hypoplastic disease of the bone marrow [1] in whom the indications for splenectomy were the presence of severe thrombocytopenic purpura anemia and granulocytopenia leading to increased susceptibility to infection improved Five of the twelve patients ranged in age from 2 to 9 years and of these two showed hematologic improvement but in only one was it sustained In another series [40] four of fifteen splenectomized patients have shown partial or complete recovery It appears that splenectomy should be reserved not only for those patients in whom a hemolytic component can be demonstrated but also for those in whom an inhibiting effect of an abnormal spleen on the marrow can be postulated The criteria for patients with completely aplastic marrows still await formulation

Miscellaneous Treatment The administration of iron is potentially harmful in patients with refractory anemias because of its increased absorption from the gastrointestinal canal supplementing the already excessive iron stores within the tissues from transfusions The use of cobalt has had many advocates with sporadic reports of beneficial effects in pure red cell anemia and other refractory anemias Cobalt has been shown to influence the production of the erythropoietic stimulating factor [19] Its possible side effects such as anorexia nausea and thyroid enlargement preclude its widespread use except under controlled conditions [3] Favorable results with cobalt however have been reported in several adults with the acquired erythrocytic hypoplasia [14 41] In our experience the results with cobalt in congenital pure red cell anemia have been uniformly disappointing

Each of the following vitamin B factors in suggested dosage has been given to patients with pure red cell anemia and other members of the hypoplastic aplastic group of anemias vitamin B_1 1 000 micrograms intramuscularly three times weekly riboflavin 10 mg three times daily nicotinamide 50 mg daily and folic acid 5 mg three times daily In the doses mentioned beneficial effects were obtained with riboflavin in the treatment of pure red cell anemia[1] and nicotinamide in hypoplastic anemia after the administration of chloramphenicol [49] Although many of these substances are known to function in specific phases of erythropoiesis their value in patients with refractory anemia is still debatable Occasionally they appear to stimulate red cell formation particularly after splenectomy

Course and Prognosis The outcome of idiopathic aplastic anemia in childhood is almost uniformly fatal The acute course of weeks and months is marked by intractable hemorrhage and infection The prognosis is better if the cause is

known and in patients with cellular rather than acellular marrows When a drug such as chloramphenicol is discontinued hematologic improvement is gradual the first sign of improvement being a decreased transfusion requirement followed by a rise in the number of neutrophils and platelets The platelets are the last of the blood elements to become normal in recovered patients[40] Cerebral hemorrhage and septicemia are the common causes of death

REFERENCES

1 Altman K I and Miller G A Disturbance of Tryptophan Metabolism in Congenital Hypoplastic Anemia, Nature 172 868 1953
1a Allen D M and Diamond L K Congenital Hypoplastic Anemia Cortisone Treated 70th Annual Meeting American Pediatric Society Swampscott Mass May 6 1960
2 Bomford R R and Rhoads C P Refractory Anemia Quart J Med 10 175 1941
3 Burgert E O Jr Kennedy R L J and Pease G L Congenital Hypoplastic Anemia Pediatrics 12 218 1954
4 Cathie I A B Erythrogenesis Imperfecta Arch Dis Childhood 25 313 1950
5 Clarkson B and Prockop D J Aregenerative Anemia Associated With Benign Thymoma New England J Med 259 253 1958
6 Dawson J P Congenital Pancytopenia Associated With Multiple Congenital Anomalies (Fanconi Type) Review of the Literature and Report of a Twenty Year Old Female With a Ten Year Follow up and Apparently Good Response to Splenectomy Pediatrics 15 325 1955
7 Diamond L K Year Book of Pediatrics 1954 1955 Series Chicago 1954 Year Book Publishers Inc p 308
8 Diamond L K and Blackfan K D Hypoplastic Anemia Am J Dis Child 56 464 1938
9 Ehrlich P Ueber einen Fall von Anämie mit Bemerkungen uber regenerative Veranderungen des Knochenmarks Charite Ann 13 300 1888
10 Eisenmann G and Dameshek W Splenectomy for Pure Red Cell Hypoplastic (Aregenerative) Anemia Associated With Autoimmune Hemolytic Disease New England J Med 251 1044 1954
11 Erslev A Hematopoietic Depression Induced by Chloromycetin Blood 8 170 1953
12 Estren S and Dameshek W Familial Hypoplastic Anemia of Childhood Am J Dis Child 73 671 1947
13 Fanconi G Familiare infantile perniziosartige Anamie (pernizioses Blutbild und Konstitution) Jahrb Kindern 117 257 1927
14 Fountain J R and Dales M Pure Red Cell Aplasia Successfully Treated With Cobalt Lancet 1 541 1955
15 Foy H and Kondi A A Case of True Red Cell Aplastic Anemia Successfully Treated With Riboflavin J Path & Bact 65 559 1953
16 Gasser C Akute Erythroblastopenie 10 Falle aplastischer Erythroblastenkrisen mit Riesenproerythroblasten bei allergischtoxischen Zustandsbildren Helvet paed at acta 4 107 1949
17 Gasser C Aplastiche Anamie (chronische Erythroblastophthise) und Cortison Schweiz med Wchnschr 81 1241 1951
18 Gasser C Aplasia of Erythropoiesis Acute and Chronic Erythroblastopenias or Pure (Red Cell) Aplastic Anemias in Childhood Pediat Clin North America May 1957 p 445
19 Goldwasser E Jacobson L O Fried W and Plzak I Mechanism of the Erythropoietic Effect of Cobalt Science 125 1085 1957
20 Hansen H G Ueber die essentielle Erythroblastopenie Acta haemat 6 335 1951
20a Havard C W H and Scott R B Thymic Tumour and Erythroblastic Aplasia Report of Three Cases and a Review of the Syndrome Brit J Haemat 6 178 1960

21 Heaton L D Crosby W H and Cohen A Splenectomy in the Treatment of Hypoplasia of the Bone Marrow With a Report of Twelve Cases Ann Surg 146 637 1957
22 Hsia D Y Y Inborn Errors of Metabolism Chicago 1959 Year Book Publishers Inc
23 Humphreys G H II and Southworth H Aplastic Anemia Terminated by Removal of Mediastinal Tumor Am J M Sc 210 501 1945
24 Josephs H W Anaemia of Infancy and Early Childhood Medicine 15 307 1936
25 Lawrence J S Dowdy A H and Valentine W Effects of Radiation on Hematopoiesis Radiology 51 400 1948
26 Lerner A B Melanin Pigmentation Am J Med 19 902 1955
27 Lescher F G and Hubble D A Correlation of Certain Blood Diseases on the Hypothesis of Bone Marrow Deficiency or Hypoplasia Quart J Med 1 425 1932
28 Lewis C N Putman L E Hendricks F D Kerlan I and Welch H Chloramphenicol (Chloromycetin) in Relation to Blood Dyscrasias With Observations on Other Drugs a Special Survey *Antibiotics & Chemother* 2 601 1952
29 Loeb V Jr Moore C V and Dubach R The Physiologic Evaluation and Management of Chronic Bone Marrow Failure Am J Med 15 499 1953
30 Martland H S Occupational Poisoning in Manufacture of Luminous Watch Dials General Review of Hazard Caused by Ingestion of Luminous Paint With Especial Reference to the New Jersey Cases J A M A 92 466 1929
31 Palmen K and Vahlquist B Stationary Hypoplastic Anemia Acta haemat 4 273 1949
32 Panels in Therapy the Use of Cobalt and Cobalt Iron Preparations in the Therapy of Anemia Blood 10 852 1955
33 Pearson H A and Cone T E Jr Congenital Hypoplastic Anemia Pediatrics 19 192 1957
34 Reinhold J D L Neumark E Lightwood R and Carter C O Familial Hypoplastic Anemia With Congenital Abnormalities (Fanconi Syndrome) Blood 7 915 1952
34a Report to the Council on Drugs Blood Dyscrasias Associated With Chloramphenicol (Chloromycetin) Therapy J A M A 172 2044 1960
35 Rhoads C P and Barker W H Refractory Anemia J A M A 110 794 1938
36 Rhoads C P and Miller D K Histology of the Bone Marrow in Aplastic Anemia Arch Path 26 648 1938
37 Robson H N and Duthie J J R Further Observations on Capillary Resistance and Adreno-cortical Activity Brit M J 1 994 1952
38 Rohr K Familial Panmyelophthisis Fanconi Syndrome in Adults Blood 4 130 1949
39 Ross J F Finch S C Street R B Jr and Strieder J M Simultaneous Occurrence of Benign Thymoma and Refractory Anemia Blood 9 935 1954
40 Scott J L Cartwright G E and Wintrobe M M Acquired Aplastic Anemia an Analysis of Thirty Nine Cases and Review of the Pertinent Literature Medicine 38 119 1959
41 Seaman A J and Koler R D Acquired Erythrocytic *Hypoplasia a Recovery During* Cobalt Therapy a Report of Two Cases and a Review of the Literature Acta haemat 9 153 1953
42 Shahidi N T and Diamond L K Testosterone Induced Remission in Aplastic Anemia A M A J Dis Child 98 293 1959
43 Smith C H The Anemias of Early Infancy J Pediat 16 375 1940
44 Smith C H Chronic Congenital Aregenerative Anemia (Pure Red Cell Anemia) Associated With Iso immunization by the Blood Group Factor A Blood 4 697 1949
45 Smith C H Hypoplastic and Aplastic Anemias of Infancy and Childhood With a Consideration of the Syndrome of Nonhemolytic Anemia of the Newborn J Pediat 43 457 1953
46 Smith C H Schulman I Ando R E and Stern G Studies in Mediterranean (Cooley s) Anemia the Suppression of Hematopoiesis by Transfusion Blood 10 707 1955
47 Smith C H Schulman I and Morgenthau J F Iron Metabolism in *Infants and Children* Serum Iron and Iron Binding Protein Diagnostic and Therapeutic Implications In Levine S Z (editor) Advances in Pediatrics Chicago 1952 Year Book Publishers Inc

48 [illegible], P. [illegible] Aplastic Anemia in Children [illegible] Aleukemia by Bone Marrow Aspiration. Pediatrics [illegible]
49 Todd, R. M. L. The Treatment of [illegible] Aplastic Anemia in Children [illegible] Dis. Childhood 29 [illegible], 1954.
50 Tsai, S. Y., and Levin, W. C. [illegible] Review of the Literature and Report of a Case. Am. J. M. [illegible]
50a Viller, R. W., Jarrold, T., [illegible], J. J., [illegible], J. F., Freeman, E. L., and [illegible] Refractory Anemia With [illegible] Bone Marrow [illegible]
51 [illegible], P., and Bassen, F. A. Sternal Marrow of Children in Normal and in Pathologic States. Am. J. Dis. Child. [illegible]
52. Vogel, P., Erf, L. A., and [illegible] Observations on Bone Marrow Obtained by Sternal Puncture. Am. J. Clin. Path. [illegible]
53 Warren, S., and Dameshek, W. (chairmen) Bone Marrow Transplantation Conference. Spec. Sect. No. 2, Blood, 13 [illegible]
54. Wintrobe, M. M. Clinical Hematology, ed. 4. Philadelphia, [illegible] Lea & Febiger.
55 Wolff, J. A. Anemias Caused by Infections and Toxins. [illegible] Aplastic Anemia Caused by Renal Disease. Pediat. Clin. North America, May [illegible]
56 Zeltmacher, K., and Bevans, M. Aplastic Anemia and Its [illegible] Hemochromatosis. Arch. Int. Med. [illegible]

21 Heaton L D Crosby W H and Cohen A Splenectomy in the Treatment of Hypoplasia of the Bone Marrow With a Report of Twelve Cases Ann Surg 146 637 1957

22 Hsia D Y Y Inborn Errors of Metabolism Chicago 1959 Year Book Publishers Inc

23 Humphreys C H H and Southworth H Aplastic Anemia Terminated by Removal of Mediastinal Tumor Am J M Sc 210 501 1945

24 Josephs H W Anaemia of Infancy and Early Childhood Medicine 15 307 1936

25 Lawrence J S Dowdy A H and Valentine W Effects of Radiation on Hematopoiesis Radiology 51 400 1948

26 Lerner A B Melanin Pigmentation Am J Med 19 902 1955

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28 Lewis C N Putnam L E Hendricks F D Kerlan I and Welch H Chloramphenicol (Chloromycetin) in Relation to Blood Dyscrasias With Observations on Other Drugs a Special Survey Antibiotics & Chemother 2 601 1952

29 Loeb V Jr Moore C V and Dubach R The Physiologic Evaluation and Management of Chronic Bone Marrow Failure Am J Med 15 499 1953

30 Martland H S Occupational Poisoning in Manufacture of Luminous Watch Dials General Review of Hazard Caused by Ingestion of Luminous Paint With Especial Reference to the New Jersey Cases J A M A 92 466 1929

31 Palmen K and Vahlquist B Stationary Hypoplastic Anemia Acta haemat 4 273 1949

32 Panels in Therapy the Use of Cobalt and Cobalt Iron Preparations in the Therapy of Anemia Blood 10 852 1955

33 Pearson H A and Cone T E Jr Congenital Hypoplastic Anemia Pediatrics 19 192 1957

34 Reinhold J D L Neumark E Lightwood R and Carter C O Familial Hypoplastic Anemia With Congenital Abnormalities (Fanconi Syndrome) Blood 7 915 1952

34a Report to the Council on Drugs Blood Dyscrasias Associated With Chloramphenicol (Chloromycetin) Therapy J A M A 172 2044 1960

35 Rhoad C P and Barker W H Refractory Anemia J A M A 110 794 1938

36 Rhoads C P and Miller D K Histology of the Bone Marrow in Aplastic Anemia Arch Path 26 648 1938

37 Robson H N and Duthie J J R Further Observations on Capillary Resistance and Adreno cortical Activity Brit M J 1 994 1952

38 Rohr K Familial Panmyelophthisis Fanconi Syndrome in Adults Blood 4 130 1949

39 Ross J F Finch S C Street R B Jr and Strieder J M Simultaneous Occurrence of Benign Thymoma and Refractory Anemia Blood 9 935 1954

40 Scott J L Cartwright G E and Wintrobe M M Acquired Aplastic Anemia an Analysis of Thirty Nine Cases and Review of the Pertinent Literature Medicine 38 119 1959

41 Seaman A J and Koler R D Acquired Erythrocytic Hypoplasia a Recovery During Cobalt Therapy a Report of Two Cases and a Review of the Literature Acta haemat 9 153 1953

42 Shahidi N T and Diamond L K Testosterone Induced Remission in Aplastic Anemia A M A J Dis Child 98 293 1959

43 Smith C H The Anemias of Early Infancy J Pediat 16 375 1940

44 Smith C H Chronic Congenital Aregenerative Anemia (Pure Red Cell Anemia) Associated With Iso immunization by the Blood Group Factor A Blood 4 697 1949

45 Smith C H Hypoplastic and Aplastic Anemias of Infancy and Childhood With a Consideration of the Syndrome of Nonhemolytic Anemia of the Newborn J Pediat 43 457 1953

46 Smith C H Schulman I Ando R E and Stern G Studies in Mediterranean (Cooley s) Anemia the Suppression of Hematopoiesis by Transfusion Blood 10 707 1955

47 Smith C H Schulman I and Morgenthau J E Iron Metabolism in Infants and Children Serum Iron and Iron Binding Protein Diagnostic and Therapeutic Implications In Levine S Z (editor) Advances in Pediatrics Chicago 1952 Year Book Publishers Inc

48 Sturgeon P Idiopathic Apla tic Anemia in Children Its Early Differentiation From Aleukemia by Bone Marrow Aspiration Pediatrics 8 216 1951
49 Todd R McL The Treatment of Hypoplastic Anaemia After Chloramphenicol Arch Dis Childhood 29 575 1954
50 Tsai S Y and Levin W C Chronic Erythrocytic Hypoplasia in Adults Review of the Literature and Report of a Case Am J Med 22 322 1957
50a Vilter R W Jarrold T Will J J Mueller J F Friedman B I and Hawkins V R Refractory Anemia With Hyperplastic Bone Marrow Blood 15 1 1960
51 Vogel P and Bassen F A Sternal Marrow of Children in Normal and in Pathological States Am J Dis Child 57 245 1939
52 Vogel P Erf L A and Rosenthal N Hematological Observations on Bone Marrow Obtained by Sternal Puncture Am J Clin Path 7 436 1937
53 Warren S and Dameshek W (chairman) Bone Marrow Transplantation Conference Spec Sect No 2 Blood 13 296 1958
54 Wintrobe M M Clinical Hematology ed 4 Philadelphia 1956 Lea & Febiger
55 Wolff J A Anemias Caused by Infections and Toxins Idiopathic Aplastic Anemia Caused by Renal Disease Pediat Clin North America May 1957 p 469
56 Zeltmacher K and Bevans M Aplastic Anemia and Its Association With Hemochromatosis Arch Int Med 75 395 1945

CHAPTER 15

The Hemolytic Anemias

Definition—General Considerations of Pathogenesis A hemolytic anemia may be defined as one due to excessive blood destruction A large number of blood disorders of varied etiology and clinical and hematologic course are included in the group of hemolytic anemias which have in common the central feature of a red cell with a reduced life span

As has been pointed out the presence or absence of anemia is dependent upon the ability of the bone marrow to respond to varying degrees of blood destruction Abnormal degrees of hemolysis therefore need not result in anemia if the bone marrow is capable of regenerating adequate amounts of hemoglobin and red blood cells [1] Bone marrow capacity has been estimated at 6 to 8 times the normal so that when the average red cell survival is reduced to 15 to 20 days instead of the normal 120 days the limit of compensation has reached its possible maximum This situation represents a completely compensated hemolytic disease without apparent evidence of anemia However if the rate of destruction exceeds the ability of the body to increase the rate of red cell production anemia will result This is termed decompensated hemolytic disease A new equilibrium is eventually re established when these forces are equalized at lower hemoglobin levels The particular concentration of hemoglobin obtained is therefore determined both by the extent of the hemolytic defect present and by the particular capacity of the bone marrow to respond with an increase in the production of erythrocytes

Classification Hemolytic anemias may be classified as congenital (hereditary) and acquired—the former related to an intracorpuscular (intrinsic) abnormality and the latter related to an extracorpuscular (extrinsic) mechanism and due to the action of external agents on structurally normal red cells A combination of factors may be present in the same patient as in patients with Cooley s anemia in whom the abnormal cells have a shortened life span within the circulation and transfused normal donor cells are destroyed by an extracorpuscular mechanism residing in the spleen (hypersplenism)

1 *Intracorpuscular or intrinsic abnormality*

A Hereditary (congenital) hemolytic anemia

(1) Hereditary spherocytosis

(2) Thalassemia

(3) Sickle cell disease
(4) Hereditary hemoglobinopathies including combinations of thalassemia and sickle cell disease
(5) Hereditary nonspherocytic hemolytic anemias
(6) Hereditary elliptocytosis with hemolytic anemia
(7) Hemolytic anemia due to enzyme deficiency apparent after administration of drugs and other agents primaquine naphthalene fava bean (favism) etc

B Nonhereditary hemolytic anemia
(1) Paroxysmal nocturnal hemoglobinuria

2 *Extracorpuscular or intrinsic mechanism*

A Acquired hemolytic anemia
(1) Due to antibodies
(a) Autoimmune antibodies
(1) Idiopathic acquired hemolytic anemias
(b) Isoagglutinins
(1) Erythroblastosis mismatched transfusion (blood or plasma)
(2) Symptomatic hemolytic anemias
(a) With Hodgkin s disease lymphosarcoma renal disease lupus erythematosus thrombotic thrombocytopenic purpura etc
(3) Paroxysmal cold hemoglobinuria
(4) Acute acquired hemolytic anemia (Lederer s anemia)
(5) March hemoglobinuria
(6) Miscellaneous causes
(a) Infections
(b) Chemical and physical agents
(c) Vegetable substances
(d) Drug sensitivity
(e) Severe burns

It is reasonable to assume then that in any patient in whom the rate of destruction of erythrocytes less than 6 to 8 times the normal no anemia need occur because of the inherent compensatory capacity of the bone marrow An exception to this principle was noted in a series of pediatric patients with minimal clinical manifestations of hereditary spherocytosis in whom anemia was present in terms of a reduced total volume of red cells (red cell mass) despite a normal concentration of hemoglobin in the peripheral blood [47] The capacity of the bone marrow was inadequate to compensate for rates of erythrocyte destruction of a magnitude less than 6 times the normal The normal peripheral hemoglobin is explained by the contracted plasma component of the circulating blood The studies of Crosby and Akeroyd [5] therefore need elaboration to determine the full range of the bone marrow s capacity under the stress of varying degrees of blood destruction

Principal Features of Increased Hemolysis The varied clinical and hematologic manifestations of a hemolytic process stem from the release of products from red cell destruction and their disposal and the compensatory activity of the bone marrow Many of these changes are observed in the urine stool and blood and are subject to quantitative measurement The evidences of increased red cell destruction to be enumerated include anemia structural abnormalities of the red cells and increased pigment output

Hemoglobinemia and Hemoglobinuria The concentration of hemoglobin nor

mally found in the plasma is less than 5 mg per 100 ml and results from red cell destruction taking place within the cells of the reticuloendothelial tissues In the event of severe intravascular hemolysis the concentration of plasma hemo globin usually exceeds the renal threshold of 100 to 150 mg per 100 ml and the pigment appears in the urine [3, 6] Hemoglobinuria occurs with marked hemo globinemia when the rate of destruction is exceedingly rapid and severe indi cating that hemolysis is taking place in the circulating blood Depending upon the amounts of pigment present the urine is pink red brown or almost black Hemoglobinuria can be detected by benzidine and guaiac reactions In patients with marked hemoglobinemia the plasma is red or brown The absence of hemo globinemia in patients with hemolytic disease indicates that extravascular hemoly sis is principally involved Hemoglobinemia is absent in patients with hereditary spherocytosis and hereditary nonspherocytic hemolytic anemia [3] mild hemo globinemia is present in those with sickle cell anemia and Cooley s anemia Severe grades of hemoglobinemia occur in patients with acute acquired hemolytic anemia such as Lederer s anemia

In patients with marked hemoglobinemia iron containing brownish granules of hemosiderin appear in the urine Treating the urine with acidified potassium ferrocyanide reveals the presence of the Prussian blue hemosiderin [3] The extent of hemosiderin excretion varies with the concentration of plasma hemoglobin Hemosiderinuria is a characteristic finding in patients with nocturnal paroxysmal hemoglobinuria in whom hemoglobinemia is usually present

Haptoglobin in Relation to Hemoglobinemia When intravascular hemolysis occurs the free hemoglobin in the plasma combines with a specific serum protein haptoglobin (Hp) comparable to the union of copper with ceruloplasmin and of iron with siderophilin or transferrin Haptoglobin is an alpha mucoprotein 1 molecule of which is capable of binding 2 molecules of hemoglobin [9] In adults the haptoglobins are present in amounts sufficient to bind up to 150 mg of hemo globin per 100 ml of serum [9] The haptoglobin hemoglobin complex is removed from the plasma by the reticuloendothelial system where the hemoglobin is degraded into bilirubin

The large molecular weight of the haptoglobin hemoglobin complex (310 000) *prevents its passage into the urine Hemoglobinuria does not occur unless the* amount of hemoglobin liberated intravascularly exceeds the binding power of the haptoglobin and the reabsorption capacity of the tubules Unbound free hemoglobin is also oxidized into methemoglobin and methemalbumin which are also eliminated into the urine It has been demonstrated by starch electrophoresis that normal serum contains three haptoglobins These are genetically con trolled[110, 110a] and one or more may be present in the serum of a single person The concentration of haptoglobin is increased in patients with infections and malignancy and in those being given steroid therapy and it is decreased or even absent in patients with increased hemolysis [9a] A reduced haptoglobin and therefore decreased hemoglobin binding may account for increased met hemalbumin formation and in certain cases for increased hemoglobinuria

Haptoglobins cannot be demonstrated in cord blood or in the early neonatal period They appear initially at the end of the first or second week of life [3, 110a]

Methemalbumin and Methemalbuminemia Methemalbumin is formed in the plasma by the union of hematin with albumin when excessive amounts of hemoglobin are released in the course of severe red cell destruction [1] Like haptoglobin it serves as a means of transporting and neutralizing products of hemolysis in this case hematin in the blood stream When hemoglobinemia is intense the spectroscopic picture of methemalbumin is distinct In lesser amounts it can also be differentiated from methemoglobin and allied compounds by the effect of chemical reagents on their "a" bands The large sized molecule prevents its passage through the kidney and its presence in the urine

Hyperbilirubinemia and Jaundice The extent of bilirubinemia depends upon the degree of hemolysis and the capacity of the liver to remove the pigment from the plasma and excrete it in the bile In the infant in the first week of life the excretion of bile is retarded by the inability of the immature liver to conjugate indirect bilirubin because of enzyme deficiency In the older patient the functional capacity of the liver may be impaired by the coexisting anemia Usually the liver is able to excrete increased amounts of bilirubin so that the degree of hyperbilirubinemia may not reflect the amount of blood destruction In patients with hemolytic anemia the plasma bilirubin ranges from 1 to 3 mg per 100 ml and is occasionally normal so that the absence of jaundice does not necessarily eliminate a diagnosis of hemolytic anemia

Urobilinogen Excretion The liver is able to excrete large amounts of bilirubin unless its function is impaired by severe anemia and anoxemia associated with the hemolytic process Only small quantities of urobilinogen are excreted by the kidneys Increased blood destruction is associated with excessive urobilinogen excretion in the feces and frequently in the urine With the limitations already cited elevated fecal urobilinogen may be considered as evidence of hemolytic disease but the amount present in the urine is not a reliable measure of this process

Spherocytosis In addition to spherocytes which characterize the blood picture of hereditary spherocytosis acquired types appear in hemolytic disease which are morphologically identical with the former The presence of spherocytes may either indicate the production of intrinsically defective erythrocytes or reflect damage to normal cells by extrinsic factors Spherocytes appear in patients with ABO erythroblastosis fetalis hemolytic anemias due to drug sensitivity and idiopathic acquired hemolytic anemia In the latter spherocytes are sometimes more numerous than in patients with hereditary spherocytosis The acquired type of spherocyte represents a normal red cell whose surface has been damaged causing irreversible contraction with an increase in osmotic and mechanical fragility Spherocytes occur in so many hemolytic anemias of varied etiology that they are not diagnostic of any single one of them

Schistocytes Red cell fragments as well as shrunken and distorted red cells seldom seen in normal blood are present in appreciable numbers in patients with hemolytic disease Red cell fragmentation has been described following severe burns[63] and exposure to chemicals

Erythrophagocytosis Erythrocytes ingested by polymorphonuclear neutrophils and monocytes are rarely found in normal blood smears but appear in those of

patients with hemolytic anemias such as erythroblastosis acquired hemolytic anemia leukemia chemical poisoning and anemias associated with infections

Heinz Bodies Heinz bodies are red cells damaged by toxic substances leading to the appearance of intracorpuscular particles and larger rounder bodies A variety of drugs such as phenylhydrazine potassium chloride naphthalene primaquine and related compounds have been implicated in their formation They are best observed in wet unstained preparations or with the brilliant cresyl blue technique for reticulocyte staining

Evidence of Increased Marrow Activity In achieving equilibrium red cell destruction is accompanied by graded increases of red cell production The latter is marked by a compensatory hyperplasia of the bone marrow resulting in proliferation of all types of nucleated red cells ranging from the early pro erythroblasts to the most mature normoblast Also included in the blood picture of hemolysis are reticulocytosis normoblastemia macrocytosis polychromatophilia polymorphonuclear leukocytosis and increased numbers of platelets

Reticulocytosis The appearance of nucleated red cells and reticulocytes in the blood smear indicates the premature delivery of red cells into the circulation Reticulocytes usually range between 0 5 and 2 per cent in the period beyond early infancy They are increased in patients with hemolytic anemia and hereditary spherocytosis and in those with acquired hemolytic anemia they may number approximately 20 to 50 per cent An increase of reticulocytes does not always serve as an accurate index of red cell production since bone marrow hyperplasia may be associated with only slight increases in reticulocytes as seen in patients with sickle cell anemia and severe Cooley s anemia Reticulocytopenia has been noted during a hemolytic (not aplastic) crisis in patients with acute immune hemolytic anemia This is explained by the suppressive action of the autoimmune mechanism on the proliferating erythroid cells In the aplastic crisis reticulocytes disappear from the peripheral blood and nucleated cells from the bone marrow Thrombocytopenia and leukopenia are accompanying features The aplastic crisis is observed more frequently than the better known hemolytic crisis and is particularly common in patients with hereditary spherocytosis sickle cell anemia and Cooley s anemia

Normoblastemia The more anemic the patient and the higher the reticulocyte count the more likely are normoblasts to appear in the peripheral blood They are particularly noticeable in patients with erythroblastosis fetalis and severe autoimmune hemolytic anemia and after splenectomy in those with Cooley s anemia

Siderocytes Siderocytes are erythrocytes containing one or more granules of ferric iron which give a positive *Prussian blue reaction for iron They are* increased in the peripheral blood of patients after splenectomy [4] as well as in patients with certain types of hemolytic anemia These include erythroblastosis fetalis hereditary spherocytosis and autoimmune hemolytic anemia Siderocytes appearing in the peripheral blood in patients with hemochromatosis reflect excessive red cell storage of iron [53]

Sideroblasts Sideroblasts are the precursors of siderocytes in the peripheral blood Siderotic granules appear in the normoblasts of patients with normal bone

marrow They are diminished in patients with iron deficiency states[7] and are exceptionally abundant in those with hemolytic disorders severe Cooley s anemia megaloblastic anemia and lead poisoning

Macrocytosis An increased number of reticulocytes in the peripheral blood and macronormoblasts in the bone marrow account for a macrocytic blood picture in patients with hemolytic disease

Increased Erythrocyte Protoporphyrin and Coproporphyrin The heme portion of hemoglobin is a ferrous protoporphyrin for whose synthesis porphyrin precursors and iron are required The porphyrins are pigments whose structural unit consists of four pyrrole rings linked by four methene bridges They differ depending upon the nature of the side chains attached to the eight free corners of the four pyrrole nuclei Thus hemoglobin myohemoglobin cytochrome and catalase represent porphyrin protein compounds Most porphyrins exist in the form of metal complexes (metalloporphyrin)—for example hemoglobin and chlorophyll in which porphyrins are combined with iron and magnesium respectively Coproporphyrin uroporphyrin and protoporphyrin are the most common porphyrins occurring naturally

Porphyrinuria describes a condition in which there is increased urinary excretion of coproporphyrin as in patients with liver disease hemolytic anemia and lead poisoning Porphyrias are rare metabolic disorders in which abnormal amounts and kinds of porphyrins are excreted in the urine and feces[8]

Congenital porphyria is a very rare metabolic disease inherited as a recessive character whose clinical features appear in infancy and childhood It is characterized by sensitivity of the skin to sunlight resulting in vesicles and bullae (hydroa aestivale) hirsutism red urine red or purplish brown staining of the teeth an enlarged spleen and a hemolytic anemia Scarring occurs on the crusts of the vesiculobullous lesions and in other exposed areas

The biochemical defect is ascribed to the failure to convert uroporphyrin formed in excessive amounts to coproporphyrin Uroporphyrin and coproporphyrin are the predominant porphyrins excreted The continuous deposition of uroporphyrin in tissues dentine and the skeleton accounts for the hypersensitivity and pigmentation of bones and teeth

Treatment consists of shielding the patient against sunlight Splenectomy which is indicated with increased blood destruction reduces the concentration of uroporphyrin and coproporphyrin in red cells plasma urine and feces The plasma porphyrins prior to splenectomy are probably derived from the destruction of circulating red cells containing these substances

Human erythrocytes contain free coproporphyrin (up to 2 micrograms per 100 ml of red blood cells) as well as free protoporphyrin (normal 15 to 60 micrograms per 100 ml of red blood cells) In patients with iron deficiency anemia and lead poisoning marked increases of protoporphyrin occur Moderate increases are found in patients with hemolytic anemias and aregenerative anemias In persons with hemolytic anemias there is a relatively greater increase in the erythrocyte coproporphyrins than in protoporphyrins The presence of large amounts of uroporphyrin and coproporphyrin in the normoblasts of the bone marrow has suggested that these substances are formed in the marrow Erythropoietic porphyria therefore has been applied as an alternative designation to congenital porphyria

Additional Tests for Detecting Abnormal Hemolysis The vulnerability of the red cells to hemolysis may be determined by a few well defined tests

Osmotic Fragility Test The resistance of the red corpuscles to varying dilutions of hypotonic sodium chloride solution constitutes an important laboratory procedure in the diagnosis of the anemias and is especially useful in the differentiation of the hemolytic group The response of blood cells in the fragility test does not necessarily reflect their reaction within the circulating blood where conditions of isotonicity prevail It constitutes however a useful gross index of the relative thickness of the major number of red cells in the sample of blood to be tested If the red cell envelope is small in relation to its volume as in the thick red cells of patients with hereditary spherocytosis the resistance is decreased Increased resistance to hemolysis is noted when the bulk of red blood cells possess relatively large surfaces in comparison with their substance as occurs in the thin red cells of patients with thalassemia major and iron deficiency anemia

Increased fragility of the red cells in hypotonic solutions of sodium chloride as compared with the fragility of normal blood occurs not only in persons with hereditary spherocytosis but occasionally in those with acute hemolytic anemia associated with infection and ingestion of drugs Decreased fragility with complete hemolysis in solutions below 0.3 per cent sodium chloride characterizes the blood of patients with sickle cell anemia mild and severe forms of thalassemia and iron deficiency

METHODS Osmotic resistance may be measured by placing small samples of blood in solutions of progressively diminishing concentrations of sodium chloride After the solutions have been allowed to stand for a prescribed time the points of minimal and maximal hemolysis are noted Hemolysis normally *begins in 0.42 per cent solution and is complete in 0.33 to 0.30 per cent*

An alternative and more precise method is the photoelectric determination of the degree of hemolysis in which the differences in the degree of hemolysis occurring in successive dilutions of hypotonic salt solution are plotted[11]

The red cells in these solutions absorb water and grow progressively larger and more rounded until their membrane is stretched to the point of rupture when hemoglobin is liberated Red cells already rounded as those in patients with autoimmune acquired hemolytic anemia and hereditary spherocytosis begin to hemolyze at more concentrated levels than normal A thinner than normal red cell absorbs more water before rupture of the red cell membrane occurs

OSMOTIC FRAGILITY AFTER INCUBATION The more rapid increase in the osmotic fragility of red cells after incubation for twenty four hours at 37 C is useful in detecting the asymptomatic subject with hereditary spherocytosis whose red cells show a normal fragility at room temperature This method is especially applicable to the parent of a child with overt evidence of hereditary spherocytosis in whom a hereditary pattern could not otherwise be elicited[13a]

Screening Fragility Test Using Finger Tip Blood A screening test which has been shown to offer information of a decisive diagnostic character employs blood from the finger and three dilutions of hypotonic solution of sodium chloride—0.375 per cent 0.35 per cent and 0.325 per cent The method as outlined here is not meant to replace the routine fragility test but serves as a gross method in screening patients suspected of having thalassemia minor or major Since blood from the finger is used controls are readily available and in *normal persons*

the degree of hemolysis in the three tubes is as follows: with 0.375 per cent solution of sodium chloride, 3 plus; with 0.35 per cent, 3 or 4 plus; and with 0.325 per cent, 4 plus.

Two or three drops of blood from the finger, depending upon the severity of the anemia, are obtained by deep puncture and then added to each of the three tubes, each containing 2 ml. of solution of the prescribed concentrations of sodium chloride. After thorough mixing of the blood and solution, the tubes are allowed to stand in a refrigerator until sedimentation has occurred. The degree of hemolysis and the residue of unhemolyzed red cells are compared with the same factors in the blood of a normal control treated in the same manner. If any clotting occurs, the tube is centrifuged at low speed for a few minutes, and the degree of hemolysis in the supernatant fluid is compared with that found in the corresponding tube of the control. Additional tubes with 0.45 and 0.425 per cent of sodium chloride will detect the increased osmotic fragility of the blood from patients with hereditary spherocytosis. These simplified tests provide prompt information and are designed for screening.

Autohemolysis Test. Normal defibrinated blood incubated under sterile conditions at 37° C. shows minimal lysis at the end of forty-eight hours (not more than 5 per cent). In blood from patients with hereditary spherocytosis and paroxysmal nocturnal hemoglobinuria, the rate of autohemolysis is greatly accelerated. In general the rates of hemolysis parallel the degree of increased fragility before incubation.[9] Selwyn and Dacie[171] reported that spontaneous autohemolysis in patients with hereditary spherocytosis is not due to progressive swelling of the cells but to a defective cell membrane which undergoes degenerative changes more rapidly than normal. They also found that autohemolysis of the red cells of patients with hereditary spherocytosis was markedly retarded when appreciable amounts of glucose were added to the blood before incubation. Abnormally rapid autohemolysis is also found in patients with other types of spherocytosis and nonspherocytic hemolytic anemia.[2]

Investigation of Red Cell Survival in Patients With Hemolytic Disorders. The use of radioactive sodium chromate for labeling red cells provides a convenient method for estimating red cell survival. Since the isotope (Cr^{51}) remains attached to the erythrocyte for considerable periods of time, the life span of the patient's red cells can be determined within his own circulation or in a normal recipient. By thus tagging the patient's red cells with Cr^{51}, it is possible not only to measure the rate of hemolysis but also to demonstrate the principal site at which this occurs. This is accomplished by reinjecting the patient's own Cr^{51}-labeled red cells and measuring the selective uptake of radioactivity over the heart, liver and spleen. A shortened survival of labeled red cells in the circulation and a much greater radioactive count over the spleen as compared with the liver suggest that the patient would benefit from splenectomy.[6] Not only autogenous cells but also the shortened survival of normal donor cells in patients with the refractory hemolytic anemias such as severe Cooley's anemia may be accompanied by a progressive increase of radioactivity over the spleen. This finding incriminates the spleen as the site of excessive hemolysis for donor cells and serves as one of the criteria for splenectomy.

Aplastic Crisis in Hemolytic Anemia. In the study of the course of hereditary spherocytosis, Owren observed that the crisis which characterize this disease was not associated with an increase in hemolysis but with a sudden cessation of erythropoiesis: reticulocytopenia, increased pallor rather than jaundice, a de-

creased concentration of serum bilirubin and varying degrees of leukopenia and thrombocytopenia Instead of an increased hyperplasia the bone marrow was depressed especially with respect to erythropoiesis Spontaneous recovery was associated with the rapid regeneration of erythropoietic tissue marked reticulocytosis leukocytosis and an increase in platelets The crises are self limited lasting one to two weeks Owren s conclusion that the crises in patients with hereditary spherocytosis are aplastic rather than hemolytic has been amply confirmed and observed in patients with other hemolytic anemias There is no uniform causative factor for the crisis although infection is regarded as the most common agent

Aplastic crises have been described in children suffering from sickle cell anemia[13,10] acquired hemolytic anemia[38] and paroxysmal nocturnal hemoglobinuria Transient aplastic or hypoplastic states are occasionally observed in patients with severe Cooley s anemia during infection and often without a *precipitating illness*[66] An infectious etiology explains the occurrence of aplastic crises within a brief period in several members of families with sickle cell anemia [9] A transitory disappearance of normoblasts from the bone marrow has been described in allergic children as a result of infection due to sensitivity to a drug [7]

It is obvious that the severity of the anemia during and following the aplastic crisis is correlated with the life span of the red cells characterizing the anemia The degree of anemia would be less marked in a person whose red cells had a normal life span of 120 days as compared with the short life span of the spherocyte or sickle cell In the patient with sickle cell anemia an acute cessation of red cell production for a few days can result in a serious drop in hemoglobin and red cells In a patient with infectious mononucleosis in whom the life span of the red cell is normal transitory erythroblastopenia was followed by minimal anemia[13] Such minor degrees of anemia in previously nonanemic persons are readily remedied on the resumption of erythropoiesis

Less comonly a true hemolytic crisis is encountered in patients with sickle cell anemia as well as in those with the more common aplastic crisis It is *characterized by a severe pain in the back abdomen and extremities* with a significant drop in erythrocytes and hemoglobin level and by an increase in serum bilirubin and reticulocytes In this situation there is a dramatic fall in the circulating red cell volume accompanied by hyperplastic bone marrow[10]

CONGENITAL HEMOLYTIC SYNDROMES

The hereditary hemolytic syndromes many of which exhibit an intrinsic abnormality of the red cell hemoglobin or both include a large variety of disorders They embrace hereditary spherocytosis hereditary elliptocytosis hereditary nonspherocytic hemolytic anemia Cooley s anemia sickle cell anemia and many of the hereditary drug induced hemolytic anemias These erythrocytic abnormalities differ from each other morphologically and when this is not obvious they are distinguished by specific laboratory tests The genetic patterns also serve as diagnostic aids Paroxysmal nocturnal hemoglobinuria *due to an inherent*

red blood cell defect is neither congenital nor hereditary. Sickle cell disease and thalassemia will be considered with the hereditary hemoglobinopathies in Chapter 16.

Hereditary Spherocytosis (Congenital Hemolytic Jaundice, Congenital Hemolytic Anemia, Spherocytic Anemia, Chronic Acholuric Jaundice, Chronic Familial Jaundice)

The designation hereditary spherocytosis emphasizes two important elements of the disease without excluding cases in which anemia and jaundice are absent. Hereditary spherocytosis is one of the four chief congenital hemolytic syndromes which exhibit a hereditary abnormality of the red cell, the others being hereditary elliptocytosis, hereditary nonspherocytic hemolytic anemia, Cooley's anemia, and sickle cell anemia.

Definition Hereditary spherocytosis is a genetically determined chronic hemolytic disease characterized by spherocytes, increased osmotic fragility of the red cells, and frequently splenomegaly.

Inheritance and Race The disease is found chiefly among Caucasians and rarely among Negroes.[9, 3] The defect is transmitted by either parent as a mendelian dominant, so that males and females are equally affected. Discrepancies in the hereditary transmission which are difficult to explain are observed. In one series only 24 per cent of the offspring of families with an affected parent showed spherocytosis instead of the expected 50 per cent.[10] Affected children have also been observed, both of whose parents possessed no clinical or hematologic evidence of this disease. Such exceptions are explained on the basis of doubtful paternity, mutation, or incomplete expressivity of the gene for hereditary spherocytosis.[9] Occasionally one of the apparently normal parents will reveal the red cell defect of spherocytosis by increased osmotic fragility or autohemolysis in excess of the normal after incubation of a blood sample at 37° C for twenty-four to forty-eight hours.

Etiology and Pathogenesis The basic defect in patients with hereditary spherocytosis consists of the production of spherocytes which, because of their shape, are trapped in the spleen and are destroyed at a more rapid rate than normal. The pathogenesis of the abnormal shape of the spherocyte has been investigated with respect to its metabolic derangements and the effect of stasis within the spleen. The spheroidal configuration is not present in the corresponding nucleated red cells in the bone marrow but appears in the reticulocytes and is established thereafter.[10] Less often a definite but slight reduction in size has been detected in the late normoblast.[35] Once having gained maturity in the circulation, the non-nucleated red cell is smaller and thicker than the corresponding normal red cell.

Extensive studies of various metabolic alterations in the spherocytes have been conducted. Disturbances in carbohydrate metabolism in these cells, leading to slow regeneration of high energy phosphate bonds, have been demonstrated, but the relationship of these findings to the shape and susceptibility to hemolysis of spherocytes is not yet clear.[9, 103, 104]

It is generally accepted that the globular shape of the red cells accelerates their removal from the circulation and their sequestration in the splenic pulp

where they undergo lysis. This process is not dependent upon specific abnormalities of the patient's spleen since it takes place as readily in the spleen of normal persons as in that of those suffering from diseases other than spherocytosis. The abnormal thickness of spherical cells does not permit them to escape readily from the pulp into the venous sinuses.[1] The stagnation of spherocytes in the spleen is apparent in the engorgement of the pulp with red cells, but the venous sinuses are, for the most part, inconspicuous and contain few red cells.[1, 9] Increasing evidence has confirmed the hypothesis that many of the erythrocytes having been trapped in the spleen undergo lysis and others are so conditioned that they are incapable of prolonged survival in the circulation after release from the spleen.[4, 1] Additional evidence that the spherocytes are primarily involved in the pathogenesis is the fact that removal of the spleen relieves the anemia, but the spherical red cells persist and their properties of increased osmotic and mechanical fragility remain unaltered.

Clinical Features. The clinical manifestations are extremely variable, particularly with regard to the time of onset and intensity of symptoms. In individual patients and among members of the same family they vary widely at different times in the course of the disease. Jaundice, symptoms referable to anemia, and splenomegaly constitute the most common clinical features of the disease. In some infants hereditary spherocytosis may be evident at birth, whereas in others symptoms may not appear until late in adult life.

Jaundice is unusual in infancy and childhood except during the newborn period, when it may simulate erythroblastosis with the same susceptibility to kernicterus in the event of hyperbilirubinemia. In the older child an icteric tint is noticeable which becomes more pronounced in the young adult and affected parent. Jaundice is accompanied by a highly pigmented urine and stool. The spleen is nearly always enlarged. In the young infant it may be palpable or may not be felt at all. Pallor with or without anemia and a sallow complexion are common. Lassitude, fatigability, anorexia, fever, and abdominal pain may call attention to the disease. Crises are ushered in by fever, extreme pallor, shortness of breath, nausea, vomiting, and extreme weakness. They are now known to be aplastic rather than hemolytic as formerly assumed and are associated with cessation of blood formation. Crises may occur in several members of the same family almost simultaneously.

Gallstones occur commonly in older persons, with an incidence as high as 85 per cent of cases;[1, 3] they are uncommon in young children but have been reported in a child 3 years old. They usually increase in frequency after the age of 10 years.[3] Gallstone colic or biliary obstruction may constitute the first indication of existing hereditary spherocytosis. A history of cholelithiasis in a parent justifies examination for spherocytosis in the offspring who is pale, easily fatigued, and without appetite. The presence of gallstones in patients with hereditary spherocytosis depends upon the activity and duration of the hemolytic process.

Leg ulcers occur infrequently and are extremely rare in childhood. Their causation is not so readily explained as in patients with sickle cell anemia. Cardiac murmurs, especially in young children, may be so intense as to simulate

congenital heart disease and lead to recognition of the underlying disease Their elimination by transfusion confirms a hemic origin Roentgenologic changes are similar to those occurring in patients with other congenital hemolytic syndromes but less in degree and incidence They most commonly consist of thickening and striation of the frontal and parietal bones of the skull [18]

Growth Among patients with hereditary spherocytosis who exhibit little in the way of symptoms but have a reduced volume of erythrocytes growth has been normal Except during transient periods of marrow hypoplasia (aplastic crisis) values for hemoglobin concentration ranged from 9 to 14 gm per 100 ml There was no deviation from the normal growth curve in any patient during the period of the expected preadolescent acceleration of growth It is during this period that patients with other chronic anemias such as thalassemia major receiving transfusion therapy fail to keep pace with rates of growth observed in normal children Thus adults with congenital spherocytosis who are relatively asymptomatic except during periods of crisis are of normal height In contrast to these patients children who are moderately or severely anemic and require repeated transfusions show impairment in both growth and weight In such patients it is undoubtedly wise to advise splenectomy at much earlier periods

Laboratory Data The increased rate of red cell destruction produces anemia hyperbilirubinemia an elevated icterus index increased fecal and urinary urobilinogen and elevated serum iron concentration The increased rate of red cell regeneration leads to reticulocytosis The blood picture is characterized by anemia spherocytosis increased osmotic fragility and an elevated serum bilirubin The Coombs test is negative and an abnormal hemoglobin is absent The hemoglobin level may be normal but usually ranges from 7 to 11 gm per 100 ml and the red blood cell count ranges from 2 5 to 4 5 million per cubic millimeter During a crisis the hemoglobin may fall to 3 to 4 gm and the red cell count to 1 5 to 2 million The reticulocytes range from 5 to 20 per cent

The spherocytes appear as small bright deeply stained cells which lack central pallor Their decreased diameter and increased thickness contribute to the rounded appearance Normoblasts may appear in the peripheral blood Poikilocytosis is rare Large polychromatophilic cells are common and appear in the blood smear in conjunction with normal and spherical cells Spherocytes may be few in number or constitute the bulk of cells Except in the mildest cases the osmotic fragility is increased When this is uncertain fragility is accelerated by prior incubation of the blood for twenty four hours at 37 C Mechanical fragility is increased The autohemolysis test reveals greater than normal lysis of the red cells during sterile incubation at 37 C for forty-eight hours The addition of glucose causes marked inhibition of autohemolysis [104,105] The leukocytes are usually normal in number or may be slightly increased The platelets are normal Bilirubinemia varies between 1 and 4 mg per 100 ml but often lies within the normal range despite other signs of active hemolysis [9]

A normal or slightly lowered hemoglobin concentration may coexist with active hemolytic disease An abnormally small total red cell volume may be present in such cases and in combination with a rapid rate of red cell production results

in a partially compensated hemolytic anemia.[4] Whether or not anemia exists depends upon the ability of red cell production to compensate for the increased destruction of cells. The failure of the plasma volume to expand accounts for the apparently normal hemoglobin level in the peripheral blood. As yet there is no known explanation for the lack of expansion of the plasma volume in patients with this disease or the greater than normal expansion in patients with sickle cell anemia.

Since hereditary spherocytosis is characterized by a chronic and persistent hemolytic process, one would expect episodes of exaggerated hemolysis to occur. In contrast to an anticipated increase in hemolysis, the crisis in hereditary spherocytosis is now known to be aplastic. Instead of a hyperplastic bone marrow which is ordinarily present in this disease, an acute aplasia of erythroid tissue supervenes and is associated with reticulocytopenia, anemia, leukopenia and thrombocytopenia. The serum bilirubin decreases and jaundice gives way to pallor.

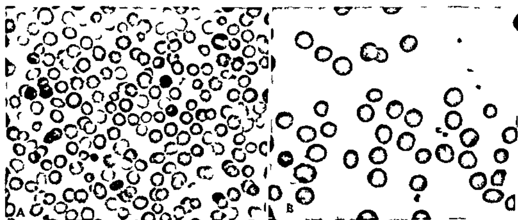


Fig. 10. Blood smear of a patient with hereditary spherocytosis. *A*, Low power view showing dark staining of abnormally thick spherocytes (×250). *B*, Slightly increased magnification of *A*.

Hereditary Spherocytosis in the Newborn Period. Hereditary spherocytosis may be manifest at birth.[96,106,113] The blood smear in the neonatal period shows both macrocytic cells from fetal life and spherocytes. The latter become increasingly numerous in the weeks following birth. Excessive jaundice due to the accumulation of indirect and unconjugated bilirubin leads to confusion with erythroblastosis. The differentiation from cases of erythroblastosis due to A and B blood groups which are also characterized by spherocytosis may be difficult. The persistence of spherocytes and a negative Coombs test in patients with hereditary spherocytosis separate the two conditions. At any rate exchange transfusions are required in patients with hereditary spherocytosis in the event of hyperbilirubinuria since the same susceptibility to nervous system damage exists as in patients with erythroblastosis.

It is noteworthy that in patients with hereditary spherocytosis several supportive transfusions may be required during the first three weeks of life be-

cause of severe anemia Thenceforth patients attain a steady state in which the anemia is minimal and symptoms are few [46]

Diagnosis The important diagnostic features of hereditary spherocytosis stem from the intrinsic red cell anomaly As has already been indicated the morphology of the red cells accounts for the increased osmotic fragility reticulocytosis jaundice and splenomegaly Spherocytosis and increased osmotic fragility are not exclusive features of hereditary spherocytosis and may be present in patients with other hemolytic disorders A positive family history and one in which relatives manifest these hematologic features contribute to the diagnosis Incubation of blood samples at 37 C for twenty four hours increases osmotic fragility in the asymptomatic and affected relative or the patient with the mild or latent disease Diagnosis may be difficult in the child without a positive family history or in one who is initially observed in an aplastic crisis

It should be emphasized that the absence of jaundice and absence of anemia are inadequate criteria for the diagnosis of a latent case or a spherocytic trait Jaundice and bilirubinemia are largely dependent upon the excretory capacity of the liver since hemoglobin and red cell levels depend not only upon the rate of hemolysis but also upon the capacity for regeneration Thus determination of fecal urobilinogen excretion may provide evidence of a marked hemolytic disorder in the absence of jaundice or hyperbilirubinemia The reticulocyte count may reveal accelerated red cell regeneration in the absence of anemia This determination is especially important in patients in whom the failure of the plasma volume to expand in the face of a diminished red cell mass results in a normal or slightly lowered hemoglobin level in the peripheral blood

Autoimmune acquired hemolytic anemia usually shows marked spherocytosis but the positive Coombs test and negative family history differentiate it from hereditary spherocytosis In patients with hereditary nonspherocytic anemia spherocytes are absent and osmotic fragility is normal

Treatment Splenectomy is followed by a complete and permanent relief from all signs and symptoms of the disease Except for the asymptomatic patient in whom red cell destruction is well compensated the operation is recommended once the diagnosis is established Even in the mildly affected person without anemia or jaundice splenectomy is advisable for the relief of a chronic hemolytic condition and proneness to crises and gallstone formation Because of evidence that splenectomy in early childhood may cause increased susceptibility to infection [11] it has been suggested that the operation be postponed until the patient is at least 5 years of age when gallstones tend to be formed [1,4] Occasionally an earlier operation at approximately 2 years of age is necessary when anorexia and fatigability seriously interfere with the health of the child By pooling data from reported sources from 1934 to 1957 of the evidence of infection following splenectomy for hereditary spherocytosis in the first year of life Burman[11] found that 10 to 21 per cent of the forty eight patients developed an infection and that 4 to 8 per cent died It would seem that splenectomy in the first year of life is definitely hazardous The optimal age for the operation still awaits the results of more extended experience Children require close supervision for several years postoper

in a partially compensated hemolytic anemia.[4] Whether or not anemia exists depends upon the ability of red cell production to compensate for the increased destruction of cells. The failure of the plasma volume to expand accounts for the apparently normal hemoglobin level in the peripheral blood. As yet there is no known explanation for the lack of expansion of the plasma volume in patients with this disease or the greater than normal expansion in patients with sickle cell anemia.

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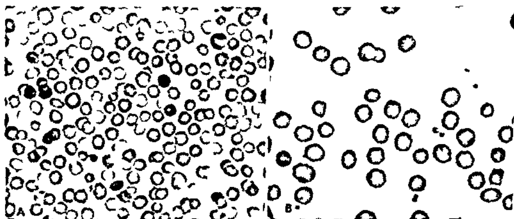


Fig. 10. Blood smear of a patient with hereditary spherocytosis. A, Low power view showing dark staining of abnormally thick spherocytes (× 530). B, Slightly increased magnification of A.

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It is noteworthy that in patients with hereditary spherocytosis several supportive transfusions may be required during the first three weeks of life be-

A familial incidence is well documented [19, 1, 103] but, as in patients with hereditary spherocytosis it can not always be demonstrated in the asymptomatic siblings or parent Sickle cell anemia Cooley s anemia and hereditary spherocytosis are readily differentiated by clinical and hematologic criteria

Increasing evidence indicates that hereditary nonspherocytic hemolytic anemia is not a single entity and that not all patients with this disease suffer from the same disorder The disorders are similar with respect to a shortened red cell survival normal osmotic fragility failure to respond to splenectomy and often development of hyperbilirubinuria and anemia shortly after birth At least two distinct types have been separated on the basis of the extent of increased osmotic fragility and autohemolysis (with or without added glucose) after twenty four hours of incubation at 37° C [*] In patients with one of these types the greatly increased osmotic fragility and the failure of glucose to diminish autohemolysis as occurs in patients with normal blood suggest a metabolic defect [*] In patients with another type the red blood cells were found to possess the properties of cells sensitive to primaquine—namely a reduced glutathione content a markedly abnormal glutathione stability test and an extreme reduction of glucose-6 phosphate dehydrogenase In patients with this type the morphology of the red cells is normal and the degree of anemia mild except during infections The defect is present in other members of the family and in several generations

Hereditary nonspherocytic hemolytic anemia should be considered in patients who present the clinical picture of erythroblastosis at birth without the characteristic laboratory findings of the latter disease [10] Hyperbilirubinemia of the indirect type in the first days of life necessitates exchange transfusion as in erythroblastosis before critical levels are reached It is characteristic however of hereditary nonspherocytic anemia that chronic anemia persists following discharge of the patient from the hospital The diagnosis is often made by exclusion from a heterogeneous variety of intrinsic blood disorders

Frequent transfusions to maintain hemoglobin levels of approximately 7 gm per 100 ml constitute the only reliable form of therapy Excessive blood requirements may lead to an extracorpuscular hemolytic defect, [4] in which case splenectomy is beneficial although the basic hemolytic process remains unaltered The congestion with spherocytes which is characteristic of the spleen of the patient with hereditary spherocytosis is strikingly lacking in the splenic pulp of the patient with nonspherocytic anemia although hemosiderin deposits are abundant

Hereditary Elliptocytosis With Hemolytic Anemia

Hereditary elliptocytosis is an uncommon hereditary anomaly characterized by the presence of large numbers of oval and elliptical cells in the peripheral blood and is usually discovered on routine examination [4] It is transmitted as a mendelian dominant and either sex may be equally affected Elliptocytes are usually found in one parent but despite this unilateral inheritance about 12 per cent of the offspring bearing the trait [9] show evidences of a hemolytic anemia In only a few reported cases have both parents been affected and in these the offspring gave evidence of a severe hemolytic anemia [8, 119] At birth elliptical red cells are few in number and do not appear in significant numbers until the third to fourth month of life although jaundice and anemia may be present in the first

atively so that immediate and energetic treatment can be instituted in the event of sudden and severe illness.

Spherocytosis as well as increased osmotic fragility of the red corpuscles persist after splenectomy. A sharp rise in platelets and leukocytes follows postoperatively. The reticulocyte count drops to normal but is occasionally slightly elevated, hemolysis ceases, and the bilirubin level falls significantly. The life span of the red cells previously shortened becomes approximately normal after splenectomy.

Blood transfusion is required in the early months of life until the hemoglobin stabilizes and for treatment of a crisis with severe anemia.

Sporadic Congenital Spherocytosis Associated With Congenital Hypoplastic Thrombocytopenia and Malformations

A syndrome characterized by congenital malformations (such as absent radii, cyanotic heart disease, and bilateral hydronephrosis), thrombocytopenia, hypoplasia of megakaryocytes, and the sporadic form of spherocytic anemia has been described in infants.[2,3] The spleen may be transiently enlarged and petechiae may be present in early infancy. A follow up into childhood is not yet available in these patients. Except for the presence of spherocytes, the combination of depleted megakaryocytes and platelets dating from the neonatal period resembles congenital hypoplastic thrombocytopenia. In many respects the clinical and hematologic features constitute a variant of Fanconi's syndrome to the extent that a severe anemia coexists with congenital abnormalities.

Hereditary Nonspherocytic Hemolytic Anemia (Atypical Familial Hemolytic Anemia, Congenital Nonspherocytic Anemia)

Hereditary nonspherocytic hemolytic anemia applies to a form of chronic hemolytic anemia, frequently familial and transmitted as a mendelian dominant, in which the red cells possess an intrinsic defect without a manifest morphologic anomaly. In the latter respect it differs from hereditary spherocytosis, sickle cell anemia, and thalassemia, in which an intrinsic defect coexists with a distinctive cellular malformation. Osmotic and mechanical resistance of the red cells is normal, and the life span of the red cells is shortened (twelve to seventeen days[91]). An abnormal type of hemoglobin has not been demonstrated. In contrast to hereditary spherocytosis, there is not only normal red cell fragility but also a failure to respond to splenectomy. The anemia varies in intensity from mild to severe, requiring transfusions. The red cells are normocytic but show a tendency toward macrocytosis, and occasionally ovalocytes are present.[1] The hematologic picture includes erythroid hyperplasia of the bone marrow, reticulocytosis, normoblasts in the peripheral blood, hyperbilirubinemia, and punctate basophilia of the erythrocytes.[4] Fecal urobilinogen is increased. The Coombs test is negative. Autohemolysis and osmotic fragility after incubation at 37 C for twenty four hours showed a slight increase over the normal. Clinical features include mild jaundice, slight to moderate enlargement of the liver and spleen, and in rare instances facial configuration of the mongoloid type. Thickening of the cranial bones and a hair on end appearance between the tables similar to that observed in patients with Cooley's anemia and sickle cell anemia have been reported.[1,93]

Diagnosis is made by the presence of elliptical cells in the patient in a parent and in other members of the family Thalassemia minor in which elliptical cells are also commonly seen may be confused with elliptocytosis [9] Elliptocytosis has been reported in association with other types of disease among them the sickle trait [9]

Treatment Patients who are asymptomatic or give evidence of compensated hemolytic disease require no treatment In addition to supportive treatment by transfusions splenectomy has benefited most patients with severe uncompensated anemia [7, 8, 9] Although red cell destruction is reduced the characteristic oval and rod shaped elliptical cells persist

Hemolytic Anemia Due to Enzyme Deficiency Following Administration of Drugs and Other Agents

The recent studies on primaquine sensitivity have brought to light intrinsic defects in red cells rendering them susceptible to hemolysis These abnormalities are apparently harmless unless red cells are exposed to primaquine and other drugs commonly used in medicine [4] It was shown that an antimalarial primaquine induced intravascular hemolysis in about 10 per cent of American Negroes but rarely in Caucasians After approximately 50 per cent of the original cells are hemolyzed the younger population of red cells is resistant to further hemolysis and the anemia is thus self limited [41]

Increasing evidence emphasizes the importance of glutathione in maintaining red cell integrity and in allowing activation of enzyme systems through its reducing or protecting action [39] The characteristics of the primaquine sensitive erythrocytes are a decreased content of reduced glutathione marked fall of glutathione after incubation of whole blood with acetylphenylhydrazine (glutathione stability test) and a deficiency of glucose 6 phosphate dehydrogenase Both in vivo and in vitro exposure of the red cell to a drug of the primaquine type causes a further fall in the already decreased glutathione content [6]

Glutathione exists in a reduced form (GSH) and an oxidized form (GS SG) The interconversion of these two is catalyzed by an enzyme glutathione reductase which requires reduced triphosphopyridine nucleotide (TPNH) for the reduction of glutathione One of the principal sources of the required TPNH (which serves as a coenzyme) is the oxidation of glucose 6 phosphate to 6 phosphogluconic acid (successive intermediates in the direct oxidation of glucose) which is catalyzed by an enzyme glucose 6 phosphate dehydrogenase It is this enzyme which is deficient in the red cells of persons sensitive to primaquine [1] When this source of TPNH is unavailable because of enzymatic deficiency the red cell is unable to maintain its glutathione in the reduced state

The glucose 6-phosphate dehydrogenase is in highest concentration in the younger cells of both sensitive and nonsensitive population and is actually higher in the very young cells of the sensitive population than in the oldest cells of the nonsensitive population The young cells of the sensitive population therefore are resistant to hemolysis since they have enough enzyme to generate sufficient TPNH to keep the glutathione reduced The defect in glutathione metabolism expresses itself in the older erythrocytes

The red blood cells of susceptible persons who have a low glutathione level show an increased tendency to form Heinz bodies The Heinz bodies are visible

month[61,119] The nucleated precursors of the elliptical cells in the marrow are round with the abnormal cells making their initial appearance in the reticulocyte stage or later No abnormal hemoglobin has been detected in the cells of patients with hereditary elliptocytosis The gene determining elliptocytosis is located in the same chromosome as that carrying the genes for the Rh blood groups[60] There is no sickling in wet preparations



Fig 11 Photomicrograph of blood smear of patient with hereditary ovalocytosis (×1200) Note larger number of elliptical oval and elongated cells

Clinical and Blood Findings The characteristic cells are oval elliptical sausage shaped elongated and rod shaped[4] and together constitute over 50 per cent of the red cell population Osmotic resistance is normal except in patients with overt hemolytic anemia in whom it is decreased The condition is usually benign without symptoms or signs of anemia notwithstanding the presence of elliptical cells in the blood In the patient with the severe hemolytic form the peripheral blood shows ovalocytes elliptocytes microspherocytes distorted microcytes and fragmented forms Small numbers of oval cells (1 to 15 per cent) may appear in the blood of normal persons[4] They are often found in increased numbers symptomatic of disorders other than hereditary elliptocytosis Such are conditions in which anisocytosis and poikilocytosis are prominent as in patients with iron deficiency anemia mild and severe Cooley s anemia macrocytic anemia and severe anemias associated with leukemia and cancer[9]

In the uncommon hemolytic cases two grades of severity are observed one with hemolysis without anemia and the other with hemolytic disease and anemia[9] In both types splenomegaly hematologic evidences of hemolysis and bone marrow regeneration make their appearance and the life span of the red cells usually normal is shortened[83]

attacks of hemoglobinuria are marked by the passage of dark urine occurring during sleep regardless of the time of day. The disease occurs most commonly in adult life (usually the third decade), but a few cases have been described in children.[81, 98] It is due to an inherent red blood cell defect, presumably an abnormal stromal protein, which makes the cells susceptible to hemolytic factors present in both the serum and plasma of normal persons and of the patient. These factors include complement, thrombin,[1] and properdin.[5] Properdin is a natural heat-labile serum protein which in association with magnesium and complement is involved in the destruction of bacteria and viruses.[9] The exact mode of action of these factors in hemolysis is complex and not yet established. The coagulation system also appears to be involved in some way in the precipitation of a hemolytic crisis. The red cells show a shortened survival when injected into normal subjects. Markedly reduced erythrocyte acetylcholinesterase activity which is localized to the stroma has been demonstrated in patients with this disease. This impairment possibly diminishes the integrity of the red cell.[16]

The patients are persistently anemic and slightly icteric and show a slight to moderate enlargement of the liver and spleen. They are fatigued, complain of headaches and abdominal and lumbar pain, and are febrile during a hemolytic crisis. Hemoglobinuria, hemoglobinemia, and hemosiderinuria, which characterize the disease, are evidence of intravascular hemolysis.

The blood picture reveals an anemia of varying severity with moderate reticulocytosis (10 to 20 per cent), polychromasia, and macrocytosis. There is a tendency toward neutropenic leukopenia and thrombocytopenia. Spherocytes are not present and osmotic fragility is not increased. The bone marrow is hypercellular, indicative of increased regeneration, and rarely aregenerative. The serum bilirubin does not exceed 3 mg. per 100 ml. A tendency toward thrombosis in the systemic (including cerebral) and portal circulations accounts for varied symptomatology. The increased rate of hemolysis produced by lowering the pH of the blood has suggested that the mechanism of the disease is related to an accumulation of acid metabolites during sleep, inducing hemoglobinuria.

In the differential diagnosis, the lack of a history of cold precipitating hemolysis eliminates paroxysmal cold hemoglobinuria. Acute acquired hemolytic anemia (Lederer's anemia), in which hemoglobinuria is also present, occurs commonly in children and is unaccompanied by pancytopenia or a congenital defect of the red cells.

The course is chronic, of varying severity, with occasional exacerbations often precipitated by infections. Death from anemia, thrombosis, and infection may occur. Prolonged survival, as well as spontaneous cures, has been reported.

The diagnosis is confirmed by the Ham acid serum tests.[6] In a positive test the patient's cells undergo hemolysis in acidified normal serum at 37° C. Additional lysis of patient's cells can be achieved by addition of thrombin to the test.

There is no specific treatment. Splenectomy is disappointing and potentially hazardous. Transfusion is the most beneficial form of treatment. Because of the possible hemolytic effect of plasma factors on the patient's erythrocytes, washed red cell suspensions have been employed with good results.

manifestations of oxidative damage to either hemoglobin and/or the stroma of the sensitive cells.[1] The chemical relationships between the drug and the oxidation of glutathione and between the oxidation state of the glutathione and hemolysis are not clear.

Similar mechanisms account for hemolytic anemia from exposure to fava beans,[116] naphthalene,[1,9] and vitamin K analogues.[1, 14] Cells sensitive to primaquine have also been shown to be unusually susceptible to hemolysis by certain aniline derivatives including sulfanilamide, phenacetin, Promizole, and acetanilid.[80] The fact that erythrocytes of sensitive persons are more susceptible to the in vitro Heinz body forming action than are cells of nonsensitive persons serves as a valuable means of predicting primaquine sensitivity.

A high proportion of asymptomatic parents and siblings of the affected person with a history of favism or hemolytic anemia due to drugs have a low glutathione content in their red cells[116] and a deficiency of glucose-6-phosphate dehydrogenase.[84] In a survey of randomly selected Negroes the incidence of persons sensitive to primaquine based on the glutathione stability test was found to be about 14 per cent among males and 2 per cent among females.[1] Among relatives of "reactors" males also predominated. The defects are apparently transmitted by a sex-linked gene with incomplete dominance.

The defect in glutathione metabolism has been thoroughly investigated in relation to naphthalene-induced hemolytic anemia in infants and children.[39, 1, 8] Only a small percentage of children who have ingested naphthalene moth balls develop a hemolytic anemia and these are most often Negroes. Abnormal numbers of Heinz bodies also appear in the cells after incubation. The hemolytic effect is attributed not to naphthalene itself but to its derivatives. Transplacental passage of these compounds was noted in one case[1, 8] in which both the mother and the newborn infant showed evidence of a hemolytic anemia after the mother ingested moth balls.

A similar mechanism (glutathione instability) has been postulated for hemolysis occasionally encountered in newborn infants who are given excessive amounts of vitamin K at birth.[1]

From the group of diseases classified as hereditary nonspherocytic hemolytic anemia one variety has also been found to possess the properties of primaquine-sensitive erythrocytes. These cells show a reduced glutathione content, marked glutathione abnormal glutathione stability, and an extreme reduction of glucose-6-phosphate dehydrogenase. In this group similar defects also appeared in the families of these patients.[10, 11] That a familial defect is not always in evidence was noted in a patient with congenital nonspherocytic hemolytic anemia in whom the disease manifested itself in the absence of Gantrisin but was accentuated by the ingestion of this drug.[94]

Nonhereditary Hemolytic Anemia—Paroxysmal Nocturnal Hemoglobinuria (Marchiafava-Micheli Syndrome)

Paroxysmal nocturnal hemoglobinuria is an uncommon type of chronic hemolytic anemia with an insidious onset and is characterized by attacks of hemoglobinuria occurring mainly at night. It is neither congenital nor hereditary. The

antibodies In patients with the hemolytic anemias caused by the agents enumerated previously they are only irregularly found In persons with autoimmune hemolytic anemia however these antibodies play a dominant role in pathogenesis and constitute one of its major features

Autoimmune Hemolytic Anemia (Chronic Idiopathic Autoimmune Hemolytic Disease, Chronic Acquired Hemolytic Anemia)

Autoimmune hemolytic anemia is characterized by the presence of demonstrable abnormal antibodies in the blood which are produced by the body and are directed against its own red cells The causation may be unknown and hence be idiopathic or symptomatic of an underlying disorder The latter disorders include chronic leukemia (mainly chronic lymphocytic leukemia) the lymphomas (Hodgkin s disease follicular lymphoblastoma reticulum cell sarcoma and lymphosarcoma) disseminated lupus erythematosus and other collagen diseases (scleroderma polyarteritis nodosa dermatomyositis) neoplasms and liver disease Autoimmune hemolytic anemia also occurs with virus pneumonia in the second or third week of illness and after the onset of infectious mononucleosis

Clinical Features Autoimmune hemolytic anemia occurs at all ages and has been reported in an infant 5 months old [2] The disease is usually chronic with an insidious onset and is prone to exacerbations of severe hemolytic anemia In children pallor lethargy fatigue anorexia and low grade fever are common Mild to moderate jaundice is present in about 75 per cent of the patients The spleen is usually palpable and varies in size when it is greatly enlarged an underlying disease should be suspected Moderate enlargement of the liver is frequent and lymphadenopathy is absent Purpura is indicative of a secondary condition Hemoglobinuria is unusual and is an occasional accompaniment of a hemolytic episode

A type of autoimmune hemolytic anemia has been described in which a depression of red cells and reticulocytes in the peripheral blood is associated with a temporary disappearance of erythroblasts from the bone marrow [44, 8] This is explained by antibodies acting peripherally on erythrocytes and reticulocytes and in the bone marrow on the erythroblasts

Blood Findings The blood picture varies with the extent of the anemia and the degree of bone marrow compensation The hemoglobin usually ranges between 10 and 11 gm per 100 ml but may drop to levels of 3 to 5 gm per 100 ml in an acute attack The blood smear shows a persistent reticulocytosis (up to 60 per cent in severe hemolytic episodes) polychromatophilia microspherocytosis anisocytosis and often a tendency toward macrocytosis Spherocytosis is marked in the active phase and less noticeable when the disease is quiescent Normoblasts appear in small numbers Erythrophagocytosis by monocytes and sometimes by neutrophils may be seen in the blood smear and in greater numbers from the buffy coat of incubated venous blood [1, 9] The polynuclear leukocyte count varies during chronic periods and may be greatly elevated during an exacerbation In one of our patients the total leukocyte count rose to 94 300 per cubic millimeter The platelet count is usually normal but may be depressed Thrombopenia has been inter

ACQUIRED HEMOLYTIC ANEMIAS

The acquired hemolytic anemias embrace a group of heterogeneous disorders of varying etiology whose pathogenesis is dependent on an extracorpuscular mechanism rather than on an intrinsic defect of the red cell. They are classified as immune or nonimmune depending upon whether or not an immune mechanism can be demostrated. The immune group comprises those conditions in which antibodies contribute to the pathogenesis. They include autoimmune hemolytic anemia, erythroblastosis fetalis, transfusion reactions, and paroxysmal cold hemoglobinuria.

The nonimmune acquired hemolytic anemias appear after treatment with drugs such as quinine, phenylhydrazine and acetylphenylhydrazine, sulfonamides and arsenical compounds, after poisoning caused by chemicals such as naphthalene, benzene, and nitrobenzene, and after exposure to physical agents such as extreme heat or cold. They may follow infection by bacteria, bacterial products, viruses, and parasites. The action of these agents is characterized by direct destruction of erythrocytes or inhibition of immature forms in the bone marrow. Obviously, treatment necessitates elimination of the offending drug, chemical, or toxic agent.

Recent investigations have shown (p. 228) that such agents as sulfonamides, naphthalene, primaquine, and vegetable poisons (for example, fava bean) owe their action to a familial and intrinsic defect in the red cell which renders it susceptible to destruction. It is to be expected that other anemias in this group will eventually disclose the same relationship. In the case of favism, ingestion of even small amounts of fava bean or inhalation of the pollen from the blossom of the plant produces in sensitive persons, especially children, a sudden acute attack of hemolysis accompanied by jaundice and often hemoglobinuria. The episode usually lasts less than a week and responds to transfusions. Hemoglobinuria occurs in severely burned patients with evidences of fragmented red cells, spherocytes, and increased susceptibility to osmotic and mechanical fragility.[63] The red cells with a shortened life span appear to be destroyed intravascularly.

Malarial infection produces anemia by toxic inhibition of the marrow and actual parasitization of the red cells by the protozoa. The fulminating hemoglobinuria and severe associated clinical symptoms which occur in the condition known as blackwater fever occur in the majority of patients in the course of infection by *Plasmodium falciparum*. Infection by organisms such as *Clostridium welchii* and *Bartonella bacilliformis* (in one of its clinical forms) is characterized by severe hemolytic anemia (Oroya fever). In this infection, which occurs in inhabitants of the central zones of Peru, Colombia, and Ecuador, numerous organisms invade the blood stream, and stained smears reveal as many as 90 per cent of the erythrocytes to be heavily invaded.

The immune acquired hemolytic anemias reveal antibodies in the blood which are detected by the Coombs (antiglobulin) test. These antibodies are globulins which are elaborated by the patient through some unknown mechanism and lead to premature destruction of the red cells—hence the designation of autoimmune

hemolytic anemia with the loss of normally acquired tolerance and the development of antibodies to the patient's own red cells.[113]

The simultaneous attacks on the different blood elements in the same destructive process was emphasized by Evans and co-workers[15, 19] who observed that acquired hemolytic anemia with sensitization of the red cells is often accompanied by thrombocytopenia. Primary thrombocytopenia in turn frequently coexists with red cell sensitization with or without hemolytic anemia.

The formation of autoantibodies has also been ascribed to other mechanisms[3] an alteration of patient's erythrocytes making them foreign to his own antibody forming mechanism and thus permitting the development of anti red cell antibodies or the formation of abnormal proteins by the patient with an abnormal reaction on the part of his antibody forming tissue. False positive Wassermann and Kahn tests sometimes found in patients with this disease fit in with the latter hypothesis.[3, 7]

Destruction of Sensitized Red Cells. The spleen, liver[6] and other portions of the reticuloendothelial system contribute by as yet unknown mechanisms to the destruction of red cells coated by antibodies. Much evidence has isolated the spleen as the important organ in which sensitized red cells are filtered from the circulation, trapped and sequestered, phagocytosed and lysed.[9, 11] Spherical red cells are particularly susceptible to lysis.

Diagnosis. The diagnostic features of autoimmune acquired hemolytic anemia consist of anemia, reticulocytosis, jaundice, splenomegaly and elevated serum bilirubin with a positive Coombs test. A positive Coombs test in patients with acquired hemolytic anemia in contrast to the negative test in those with hereditary spherocytosis is helpful in differentiating the two conditions since spherocytosis and reticulocytosis occur in both. In all patients with hemolytic anemia with a positive Coombs test search should be made for one of the underlying diseases previously enumerated.

Treatment. Steroid hormones, blood transfusions and splenectomy constitute the basis of treatment.

Adrenocortical Steroids. Cases of autoimmune acquired hemolytic anemia and often symptomatic cases usually respond favorably to hormonal therapy. Permanency of improvement with these agents has not yet been thoroughly ascertained. The steroid hormones either alone or combined with splenectomy represent the treatment of choice. As with other blood disorders in which steroid hormones are used, dosage must be individualized.

Prednisone or prednisone-like compounds are most commonly given initially in a total daily dosage varying from 40 to 60 mg (for precise dosage and variety of corticosteroids see Chapter 23). When the response of the patient and the severity of the disease are ascertained, the dosage may be adjusted accordingly. In favorable cases the dose is reduced within a week. This is marked by a fall in the reticulocyte count, a rise in the number of red blood cells and hemoglobin and a reduction in the number of transfusions and their elimination. Once a remission is obtained, the steroid hormone is gradually reduced to a maintenance dose which in our experience is approximately 15 mg daily. With relapse the initial dose is restored. In spite of improvement a positive Coombs test may persist

preted as part of the immune antibody mechanism in which the red cells are intensely involved [18,19]

Osmotic fragility and autohemolysis are both increased and parallel the degree of spherocytosis An aplastic crisis indicated by reticulocytopenia leukopenia and erythrocytic aplasia of the bone marrow is rare The major fraction of increased hemolysis occurs in normal extravascular sites with the formation of *excessive amounts of bile With unimpaired liver function the bilirubin level* is not greatly elevated and usually ranges from 1 to 4 mg in quiescent periods

Serologic Findings Three different autoantibodies affect the red cells in autoimmune hemolytic anemia autoagglutinins autohemolysins and incomplete antibodies permitting agglutination by antiglobulin serum (Coombs test) The blood of patients with autoimmune hemolytic anemia commonly undergoes rapid spontaneous autoagglutination after withdrawal from the body resulting in clumping of the red cells so that blood counts are difficult The autoantibodies may be nonspecific and therefore act as panagglutinins and panhemolysins They react with normal erythrocytes in addition to the patient's own cells regardless of blood group or type In some cases the serums react specifically and usually with well recognized blood group antigens within the Rh system [37]

The antibodies may be absorbed on the surface of the red cells in which case they are detected by the direct Coombs antiglobulin test or are free in the serum Their presence in serum may be demonstrated by the indirect Coombs technique in which normal red cells are initially exposed to the patient's serum before testing with the Coombs antiglobulin reagent In neither case does the intensity of the Coombs test bear any relationship to the severity of the disease

Two types of autoantibodies can be distinguished by their behavior in vitro a warm type active at 37 C (the more common in the idiopathic autoimmune disease)[31] and the clinically *infrequent cold type active at temperatures of 4 C* High titer cold agglutinins are potentially hemolytic[30] Both types give positive direct antiglobulin (Coombs) reactions Autoagglutination by cold antibodies is abolished at 37 C Anemia reticulocytosis and spherocytosis are not as conspicuous as in the patient with the hemolytic anemia with warm agglutinins Hemolytic anemia associated with high titer cold hemagglutinins are principally found in patients with virus pneumonia In older persons they are also active in *the development of a rare form of chronic hemolytic anemia with hemoglobinuria* high titer cold hemagglutinins and Raynaud's phenomenon [16,114] Cold antibodies are also involved in the pathogenesis of paroxysmal cold hemoglobinuria The Donath Landsteiner test which is diagnostic of this disorder requires cold for the union of antibodies with the red blood cells and warmth together with complement for eventual lysis The titer of cold agglutinins is low in comparison with the elevated titer of agglutinins in patients with viral pneumonia

Pathogenesis It is of interest that the body in health avoids forming antibodies against its own tissues a tolerance developed from early fetal life [5] Such dire circumstances in which a living organism becomes capable of producing antibodies against its own body components has been termed "horror autotoxicus" by Ehrlich [118] This situation apparently exists in autoimmune

toward spontaneous recovery and relatively recent introduction of hormone therapy Fatalities have occurred in a relatively small number of children [9]

Paroxysmal Cold Hemoglobinuria

In paroxysmal cold hemoglobinuria a rare syndrome sudden attacks of intravascular hemolysis and hemoglobinuria are precipitated by exposure to cold The disorder is due to the presence of an autohemolysin in the patient s blood which is biphasic uniting with the red cells at a low temperature and destroying them in the presence of complement upon subsequent warming up to body temperature The Donath Landsteiner test demonstrates this sequence of events by bringing together cells and serum in the cold and warming the suspension to allow complement to produce hemolysis The direct antiglobulin (Coombs) test is positive at the height of a hemolytic episode and negative when the patient is symptom free [9] The Donath Landsteiner antibody causes agglutination as well as hemolysis [9]

The clinical and hematologic picture is that which characterizes acute hemolytic anemia especially when hemolysis is intravascular fever a shaking chill headache pain in the abdomen and back and the passage of dark bloody urine In addition to severe anemia and other features of hemolysis during the paroxysm leukopenia and erythrophagocytosis appear [69] the latter serving to remove damaged erythrocytes Paroxysmal cold hemoglobinuria occurs usually in patients with syphilis especially congenital syphilis Occasionally there is no clinical evidence of syphilitic infection and a positive Wassermann or Kahn test is interpreted as being false Treatment is directed toward the syphilitic infection when required and toward the avoidance of chilling Transfusion of fresh whole blood is potentially harmful because this might result in adding complement to the patient s blood and thus provoke hemolysis of his own and the transfused cells [117]

Dysproteinemias

The dysproteinemias represent a group of disorders characterized by an abnormality of the plasma proteins They include cryoglobulinemias hyperglobulinemias and macroglobulinemias (Waldenstrom s syndrome) They have in common hemorrhagic manifestations Although these conditions do not occur in the pediatric age group one of these cryoglobulinemia may be mentioned here because of its association with hemolytic anemia

Cryoglobulinemia Cryoglobulinemia refers to the presence in the serum of an abnormal globulin (cryoglobulin) which precipitates spontaneously in the cold and redissolves on subsequent warming [80,81] Cryoglobulinemia is usually secondary to another disorder such as multiple myeloma leukemia or malignant lymphomas It is associated with purpura and Raynaud s phenomenon occurring after exposure to cold Acute hemolytic anemia and high titer cold agglutinins have also been described [17]

Acute Acquired Hemolytic Anemia (Lederer s Anemia)

Sporadic cases of acute hemolytic anemia often referred to as Lederer s anemia [78] occur most commonly in children The condition is characterized

and should prompt an alertness to recurrences of the hemolytic state If prednisone or a substitute fails to achieve a remission ACTH should be tried before or after splenectomy In summary steroid hormones in adequate dosage represent a remarkably effective form of therapy especially in the initial control of this disorder[30]

Transfusions Blood transfusions are given when the hemoglobin level drops below 7 to 8 gm per 100 ml and are repeated in accordance with the demands of the patient to maintain normal activity without discomfort Transfusions represent supportive therapy but do not alter the hemolytic process Although of temporary value they play an important role in tiding the patient over a severe hemolytic episode In the severe case the transfused cells are rapidly destroyed and the hemoglobin rise is not maintained The extrinsic mechanism in this disease (the autoimmune antibodies) destroys not only the patient's cells but also transfused normal cells as well

The presence of autoantibodies frequently interferes with the typing and cross matching of blood In these cases the least incompatible blood is utilized pending the effect of steroid hormones Careful cross matching slow administration of blood and alertness to reactions are essential Occasionally it is not feasible to maintain a hemoglobin at optimal levels and the patient is required to make appropriate adjustments until other modes of therapy are introduced Replacement transfusion was successfully employed in a patient with severe hemolysis with dramatic although temporary response The purpose of this procedure was to remove large amounts of antibody and antibody coated red cells[9]

Splenectomy Prior to the advent of ACTH and corticosteroid therapy great reliance was placed on splenectomy for treatment Results were unpredictable however and the recovery rate when splenectomy was done as an initial procedure was estimated as approximately 50 per cent in patients with autoimmune hemolytic anemia Permanency in the favorable case was extremely uncertain Since autoantibodies are produced in large measure by the spleen removal of this organ would be expected to result in a sharp decrease in their concentration The variable effect of splenectomy however indicates that the spleen may not be the sole source of antibody

At present splenectomy is reserved for patients in whom steroid therapy alone is inadequate especially when excessively large doses of prednisone are required to control the hemolysis without inducing serious metabolic side effects Following splenectomy the resumption of steroid hormones is usually necessary At present steroid therapy represents the treatment of choice and splenectomy should be reserved for those patients who fail to respond to hormone therapy[14]

Prognosis The outlook in autoimmune acquired hemolytic anemia has greatly improved with the advent of steroid therapy A complete and sustained remission rate of approximately 80 per cent has been obtained with the use of corticosteroids or corticosteroids plus splenectomy[30] Severe anemia reticulocytopenia thrombocytopenia and leukopenia are associated with a high mortality rate[8] The varied forms and combinations of therapy however still await a longer experience for ultimate evaluation The effects of therapy are difficult to measure because of the uncertain course remissions exacerbations tendency

Attacks recur in the same person after brisk exercise The mechanism of hemo globinuria is still unknown Chilling of an extremity fails to produce the condition A closely allied syndrome termed "exercise myohemoglobinuria" has been de scribed in which painful swollen muscle groups and the excretion of dark urine after excessive exercise were the essential complaints [11]

REFERENCES

1 Aldrich R A Hawkinson V Grinstein M and Watson C J Photosensitive or Congenital Porphyria With Hemolytic Anemia Clinical and Fundamental Studies Before and After Splenectomy Blood 6 685 1951

1a Allison A A and ap Rees W The Binding of Haemoglobin by Plasma Proteins (Haptoglobins) Its Bearing on the Renal Threshold for Haemoglobin and the Aetiology of Haemoglobinuria Brit M J 2 1137 1957

1b Auditore J V and Hartmann R C Paroxysmal Nocturnal Hemoglobinuria II Erythrocyte Acetylcholinesterase Defect Am J Med 27 401 1959

2 Barr D P Reader G G and Wheeler C H Cryoglobulinemia Ann Int Med 32 6 1950

3 Bates G C and Brown C H Incidence of Gall Bladder Disease in Chronic Hemolytic Anemia (Spherocytosis) Gastroenterology 21 104 1952

4 Beutler E The Hemolytic Effect of Primaquine and Related Compounds a Review Blood 14 103 1959

5 Beutler E Dern R J and Alving A S The Hemolytic Effect of Primaquine a Study of Primaquine Sensitive Erythrocytes J Lab & Clin Med 44 177 1954

6 Beutler E Dern R J and Alving A S The Hemolytic Effect of Primaquine an in Vitro Test for Sensitivity of Erythrocytes to Primaquine J Lab & Clin Med 45 40 1955

7 Blackburn E K Jordan A Lytle W J Swan H T and Tudhope G R Hereditary Elliptocytic Haemolytic Anemia J Clin Path 11 316 1958

8 Blaisdell R K Priest R E and Beutler E Paroxysmal Nocturnal Hemoglobinuria a Case Report With a Negative Ham Presumptive Test Associated With Serum Properdin Deficiency Blood 13 1074 1958

9 Bowman J W Acquired Hemolytic Anemia Use of Replacement Transfusion in a Case A M A Am J Dis Child 89 -26 1955

9a Brus I and Lewis S M The Haptoglobin Content of Serum in Haemolytic Anaemia Brit J Haemat 5 348 1959

10 Britton O C Crosby W H and Motulsky A G Hereditary Nonspherocytic Hemolytic Anemia Presenting as Hemolytic Disease of the Newborn Infant Pediatrics 13 41 1954

11 Burman D Congenital Spherocytosis in Infancy Arch D Childhood 33 335 1958

12 Carson P E Flanagan C L Ickes C E and Alving A S Enzymatic Deficiency in Primaquine Sensitive Erythrocytes Science 124 484 1956

13 Chernoff A I and Josephson A M Acute Erythroblastopenia in Sickle Cell Anemia and Infectious Mononucleosis A M A Am J Dis Child 8- 310 1951

14 Chertkow G and Dacie J V Results of Splenectomy in Autoimmune Haemolytic Anaemia Brit J Haemat 2 -37 1956

15 Childs B Zinkham W H Browne E A Kimbro E L and Torbert J V A Genetic Study of a Defect in Glutathione Metabolism of the Erythrocyte Bull Johns Hopkins Hosp 102 21 1958

16 Christenson W N and Dacie J V Electrophoresis of Acquired Haemolytic Anaemia Serum Abnormal Gamma Peak Composed of Cold Antibody Protein Transactions of Sixth Congress of the European Society of Haematology Copenhagen 1957

17 Conn H O Acute Hemolytic Anemia Cryoglobulinemia and Cold Agglutination Report of a Case New England J Med 253 1011 1955

by an acute onset, short duration, and spontaneous recovery. Hemoglobinuria, jaundice, vomiting, prostration, restlessness, elevation of temperature, leukocytosis, and marked anemia are predominant features. Splenomegaly is inconsistent. Azotemia, oliguria, and anuria may be present, but death from renal failure is rare. The infrequency with which antibodies and a positive antiglobulin (Coombs) test are found is probably explained by the rapid course. The peripheral blood picture is one of a regenerative anemia with polychromatophilia, reticulocytosis, spherocytes, normoblasts, and erythrophagocytosis. Marked polynuclear leukocytosis (66,000 per cubic millimeter in one of our patients) with numerous metamyelocytes and myelocytes is indicative of increased bone marrow activity. In its subacute and milder form the disease may be difficult to differentiate from the autoimmune hemolytic disease with respect to the blood picture and serologic findings.[37, 111] The acute hemolytic episode may continue in a milder form which is undistinguishable from the autoimmune disease. When tested at appropriate times antibodies can be demonstrated in as many as 50 per cent of patients.[71]

Despite the usual self-limited course, transfusions are required to maintain adequate hemoglobin levels. In the early stages when differentiation from an exacerbation of the chronic autoimmune disease has not yet been established, ACTH and adrenocortical steroids are usually given. Regardless of the precise diagnosis, these agents are of great value at the onset of the acute fulminating disease when the anemia is severe and the patient is in a state of severe collapse.

Idiopathic Paroxysmal Myoglobinuria

Hemoglobinuria must be distinguished from myoglobinuria. Idiopathic paroxysmal myoglobinuria is a rare disease of unknown etiology in which myoglobin is liberated from muscle and appears in the urine. Myoglobinuria can result from crushing injuries of muscle or can be idiopathic and unassociated with any specific or discernible cause. Severe muscle pain, especially in the calves, thighs, and back, spasm, weakness, or complete paralysis may result with the excretion of burgundy-colored urine.[103, 111] Myoglobin, having one fourth of the molecular weight of hemoglobin, passes readily through the glomerular membrane. The disease is prone to recur and to be precipitated by exercise. Myoglobin and hemoglobin give a positive benzidine reaction. The absence of erythrocytes in the urine, the differences in the absorption bands observed by spectroscopic examination of the urinary pigment, and a negative test for porphyrin establish the diagnosis of myoglobinuria. The mortality rate is 30 per cent,[2, 4] with death caused by acute renal failure or respiratory paralysis.

March Hemoglobinuria

March hemoglobinuria is a rare and benign abnormality occurring in otherwise healthy young men in whom strenuous physical exertion carried out in the erect position is followed by the passage of dark urine.[9] Hemoglobinuria is secondary to hemoglobinemia; jaundice, anemia, and reticulocytosis are absent. The condition has been observed in soldiers after marching, in marathon runners, and in college athletes. The patient recovers spontaneously within a few months.

44 Eisenmann G and Dameshek W Splenectomy for Pure Red Cell Hypoplastic (Aregenerative) Anemia Associated With Autoimmune Hemolytic Disease New England J Med 251 1044 1954

45 Emerson C P., Jr Shen S C Ham T H Fleming E M and Castle W B Studies on the Destruction of Red Blood Cells Quantitative Methods for Determining the Osmotic and Mechanical Fragility of Red Cell in the Peripheral Blood and Splenic Pulp the Mechanism of Increased Hemolysis in Hereditary Spherocytosis as Related to the Functions of the Spleen A M A Arch Int Med 97 1 1956

46 Erlandson M E and Hilgartner M Hemolytic Disease in the Neonatal Period and Early Infancy J Pediat 54 566 1959

47 Erlandson M E Schulman I and Smith C H Studies on Congenital Hemolytic Syndromes Rates of Destruction and Production of Erythrocytes in Hereditary Spherocytosis Pediatrics 23 462 1959

48 Evans R S and Duane R T Acquired Hemolytic Anemia the Relation of Erythrocyte Antibody Production to Activity of the Disease the Significance of Thrombocytopenia and Leucopenia Blood 4 1196 1949

49 Evans R S Takahashi K Duane R T Payne R and Liu C Primary Thrombocytopenic Purpura and Acquired Hemolytic Anemia Evidence for a Common Etiology A M A Arch Int Med 87 48 1951

50 Fadem R S Ovalocytosis Associated With the Sickle Cell Trait Blood 4 505 1949

51 Fairley H N Methemalbumin Quart J Med 34 95 1941

52 Feinberg A W and Watson J Nonspherocytic Chronic Hemolytic Anemia With Basophilic Stippling Report of a Case in a Negro Blood 6 357 1951

53 Finch S C and Finch C A Idiopathic Hemochromatosis an Iron Storage Disease Iron Metabolism in Hemochromatosis Medicine 34 381 1955

54 Florman A L and Wintrobe M M Human Elliptical Red Corpuscles Bull Johns Hopkins Hosp 63 209 1938

55 Gairdner D Association of Gall-stones With Acholuric Jaundice in Childhood Arch Dis Childhood 14 109 1939

56 Gardner F H and Diamond L K Autoerythrocytic Sensitization Form of Purpura Producing Painful Bruising Following Auto Sensitization to Red Blood Cells in Certain Women Blood 10 675 1955

57 Gasser C Akute Erythroblastopenie 10 Falle aplastischer Erythroblastenkrisen mit Riesenproerythroblasten bei allergisch toxischen Zustandsbildern Helvet paediat acta 4 107 1949

58 Gasser C Aplasia of Erythropoiesis Acute and Chronic Erythroblastopenias or Pure (Red Cell) Aplastic Anaemias in Childhood Pediat Clin North America p 445 May 1957

59 Gilligan D R and Blumgart H L March Hemoglobinuria Studies of Clinical Characteristics Blood Metabolism and Mechanism With Observations on Three New Cases and a Review of the Literature Medicine 20 341 1941

60 Goodall H E Hendry D W W Lawler S D and Stephen S A Data on Linkage in Man Elliptocytosis and Blood Groups Ann Eugenics 17 267 1953

61 Gross R T Marks P A Hurwitz R E Beasley J and Sia M Studies in the Hereditary Enzymatic Defect in Certain Drug Induced Anemias A M A J Dis Child 96 436 1958

62 Ham T H (editor) A Syllabus of Laboratory Examination in Clinical Diagnosis Cambridge Mass 1950 Harvard University Press

63 Ham T H Shen S C Fleming F M and Castle W B Studies on the Destruction of Red Blood Cells—Thermal Injury Action of Heat in Causing Increased Spheroidicity Osmotic and Mechanical Fragilities and Hemolysis of Erythrocytes Observations on the Mechanisms of Destruction of Such Erythrocytes in Dogs and in a Patient With a Fatal Thermal Burn Blood 3 373 1948

64 Helz M K and Menten M L Elliptocytosis a Report of Two Cases J Lab & Clin Med 29 185 1944

18 Cooper E L Familial Acholuric Jaundice Associated With Bone Changes Ann Int Med 15 858 1941
19 Crosby W H Hereditary Non spherocytic Hemolytic Anemia Blood 5 233 1950
20 Crosby W H Paroxysmal Nocturnal Hemoglobinuria a Specific Test for the Disease Based on the Ability of Thrombin to Activate the Hemolytic Factor Blood 5 843 1950
21 Crosby W H Paroxysmal Nocturnal Hemoglobinuria Plasma Factors of the Hemolytic System *Blood 8 444* 1953
22 Crosby W H Paroxysmal Nocturnal Hemoglobinuria Relation to the Clinical Manifestations Underlying Pathogenic Mechanisms Blood 8 769 1953
23 Crosby W H The Hemolytic States Bull New York Acad Med 30 27 1954
24 Crosby W H Siderocytes and the Spleen Blood 12 165 1957
25 Crosby W H and Akeroyd J H The Limit of Hemoglobin Synthesis in Hereditary Hemolytic Anemia Its Relation to the Excretion of Bile Pigment Am J Med 13 273 1952
26 Crosby W H and Dameshek W The Significance of Hemoglobinemia and Associated Hemosiderinuria With Particular Reference to Various Types of Hemolytic Anemia, J Lab & Clin Med 38 829 1951
27 Crosby W H and Rappaport H Reticulocytopenia in Autoimmune Hemolytic Anemia Blood 11 929 1956
28 Crosby W H and Rappaport H Autoimmune Hemolytic Anemia Analysis of Hematologic Observations With Particular Reference to Their Prognostic Value a Survey of 57 Cases Blood 12 42 1957
29 Dacie J V The Haemolytic Anaemias Congenital and Acquired New York 1954 Grune & Stratton Inc
30 Dacie J V *The Auto immune Haemolytic Anaemias Am J Med. 18 810* 1955
31 Dacie J V Autoimmunization in Respect of Red Cells Program of Sixth International Congress of Hematology Boston 1956 p 488
32 Dacie J V Acquired Hemolytic Anemias Brit M Bull 15 67 1959
33 Dacie J V and Cutbush M Specificity of Auto-antibodies in Acquired *Haemolytic* Anaemia J Clin Path 7 18 1954
34 Dacie J V and deGruchy G C Auto antibodies in Acquired Haemolytic Anaemia J Clin Path 4 253 1951
35 Dameshek W Hemolytic Anemia Direct and Indirect Indications Pathogenetic Mechanisms and Classifications Seminars on the Hemolytic Anemias Am J Med 18 315 1955
36 Dameshek W and *Komninos* Z. D *The Present Status* of Treatment of Autoimmune Hemolytic Anemia With ACTH and Cortisone Blood 11 648 1956
37 Dameshek W and Schwartz S O Acute Hemolytic Anemia (Acquired Hemolytic Icterus Acute Type) Medicine 19 231 1940
38 Davis L J Kennedy A C Baikie A G and *Brown A Haemolytic Anaemias of* Various Types Treated With ACTH and Cortisone Glasgow M J 33 263 195_
39 Dawson J P Thayer W W and Desforges J F Acute Hemolytic Anemia in the Newborn Infant Due to Naphthalene Poisoning Report of Two Cases With Investigations Into the Mechanism of the Disease Blood 13 1113 1958
40 Dern R J Beutler E and Alving A S The Hemolytic Effect of Primaquine the Natural Course of Hemolytic Anemia and the Mechanism of Its Self limited Character J Lab & Clin Med 44 171 1954
41 Dern R J Beutler E and Alving A S The Hemolytic Effect of Primaquine Primaquine Sensitivity as a Manifestation of Multiple Drug Sensitivity J Lab & Clin Med 45 30 1955
42 Dresbach M Elliptical Human Red Corpuscles Science 19 469 1904
43 Dunsky I Smith F and Gibson S Porphyria and Porphyrinuria Report of a Case Review of Porphyrin Metabolism With a Study of Congenital Porphyria Am J Dis Child 74 305 1947

88 Mill r G Sl umway C N Jr and Young L E Autoimmune Hemolytic Anemia Pediat Clin North America p 429 May 195"
89 Millichap J G Acute Idiopathic Haemolytic Anaemia, Arch Dis Childhood 2" 222, 1952
90 Modell W Drugs of Choice St Louis 1958 The C V Mosby Co p "03
91 Motulsky A G Crosby W H and Rappaport H Hereditary Nonspherocytic Hemolytic Disease a Study of a Singular Familial Hemolytic Syndrome Blood 9 "49 1954
92 Motulsky A G, Singer K Crosby W H, and Smith V The Life Span of the Elliptocyte Hereditary Elliptocytosis and Its Relationship to Other Familial Hemolytic Diseases Blood 9 57 1954
93 Nelson M G Atypical Congenital Haemolytic Anaemia, Arch Dis Childhood 29 45" 1954
94 Newton W A Jr and Bass J C Glutathione Sensitive Chronic Nonspherocytic Hemolytic Anemia A M A J Dis Child 96 501 1958
95 Owren P A Congenital Hemolytic Anemia Pathogenesis of the "Hemolytic Crisis" Blood 3 231 1948
96 Pearson C M Beck, W S and Bland W H Idiopathic Paroxysmal Myoglobinuria A M A Arch Int Med 99 3"6 195"
97 Penfold J B and Lipscomb J M Elliptocytosis in Man Associated With Hereditary Haemorrhagic Telangiectasia Quart J Med 12 157 1943
98 Pierce P P and Aldrich, C A Chronic Hemolytic Anemia With Paroxysmal Nocturnal Hemoglobinuria (Marchiafava Micheli Syndrome) Report of a Case With Marked Thrombocytopenia in a Five Year Old Child J Pediat 22 30 1943
99 Pillemer L Blum L Lepow I H Ross O A Todd E W and Wardlew A C *The Properdin System and Immunity Demonstration and Isolation of a New Serum* Protein Properdin and Its Role in Immune Phenomena, Science 120 2"9 1954
100 Prankerd T A J Altman K L and Young L E Abnormalities of Carbohydrate Metabolism of Red Cells in Hereditary Spherocytosis J Clin Invest 34 1268 1955
101 Rose B S and Mabarro S N Four Cases of Acute Aquired Haemolytic Anaemia in Childhood Treated With ACTH Arch Dis Childhood 28 8" 1953
102 Scott R B In Gellis S S Yearbook of Pediatrics 195" 1958 Chicago 1958 Year Book Publishers Inc p 294
103 Segar W E Idiopathic Paroxysmal Myoglobinuria, Pediatrics 23 12, 1959
104 Selwyn J G and Dacie J V Autohemolysis and Other Changes Resulting From the Incubation in Vitro of Red Cells From Patients With Congenital Hemolytic Anemia, Blood 9 414 1954
105 Shahidi N T and Diamond L K Enzyme Deficiency in Erythrocytes in Congenital Nonspherocytic Hemolytic Anemia Pediatrics 24 245 1959
106 Shapiro C M Josephson A M Rozengvaig S and Kauffman A Hereditary Spherocytosis in the Neonatal Period J Pediat 50 308 1957
107 *Singer K Motulsky A G and Wile S A Aplastic Crisis in Sickle Cell Anemia J* Lab & Clin Med 35 "21 1950
108 Smiley R K Dempsey H Villeneuve P and Campbell J S Atypical Familial Hemolytic Anemia Blood 11 324 1956
109 Smith, C H Diagnosis of Anemias in Infancy and Childhood, J A M A 134 991 194"
110 Smith, C H Erlandson, M E Schulman I and Stern, G Hazard of Severe Infections in Splenectomized Infants and Children Am J Med 22 390 1957
110a. Smithies O Zone Electrophoresis in Starch Gels Group Variations in the Serum Proteins of Normal Human Adults Biochem J 61 629 1955
110b Smithies O and Walker N F Genetic Control of Some Serum Proteins in Normal Humans Nature 1"6 1265 1955
111 Spaet T H Rosenthal M C and Dameshek W Idiopathic Myoglobinuria in Man Report of a Case Blood 9 881 1954
112 Stahl, W C March Hemoglobinuria Report of Five Cases in Students at Ohio State University J A M A 164 13 19"7

65 Jandl J H Greenberg M S Yonemoto R H and Castle W B Clinical Determination of the Sites of Red Cell Sequestration in Hemolytic Anemias J Clin Invest 35 842 1956
66 Jandl J H and Greenberg M S Bone Morrow Failure Due to Nutritional Deficiency in Cooley s *Hemolytic Anemia Painful Erythropoietic Crises in Response to Folic Acid* New England J Med 260 461 1959
67 Jandl J H Jones A R and Castle W B The Destruction of Red Cells by Antibodies in Man Observations on the Sequestration and Lysis of Red Cells Altered by Immune Mechanisms J Clin Invest 36 1428 1957
68 Jones N C Hughes and Szur L Determination of the Sites of Red Cell Destruction Using [a] CR Labelled Cells Brit J Haemat 3 320 1957
69 Jordan W S Jr Prouty R L Heinle R W and Dingle J H The Mechanism of Hemolysis in Paroxysmal Cold Hemoglobinuria Erythrophagocytosis and Leukopenia Blood 7 387 1952
70 Josephs H W and Avery M E Hereditary Elliptocytosis Associated With Increased Hemolysis Pediatrics 16 741 1955
71 Kaplan E and Zuelzer W W Familial Non spherocytic Hemolytic Anemia Blood 5 811 1950
72 Kaplan E Zuelzer W W and Mouriquand C Sideroblasts a Study of Stainable *Nonhemoglobin Iron in Marrow Normoblasts Blood* 9 203 1954
73 Kline A H and Holman G H Hereditary Spherocytosis in the Negro A M A J Dis Child 94 609 1957
74 Kruit W Smith R T Marvin J F Read R and Good R A Congenital Non*spherocytic Hemolytic Anemia Two Nonfamilial Cases With Red Cell Survival Studies* J Pediat 49 245 1956
75 Larson A Chronic Idiopathic Autoimmune Hemolytic Disease in Children Acta pediat 46 144 1957
75a Lathem W and Jensen W N Plasma Hemoglobin Binding Capacity in Sickle Cell Disease Blood 14 1047 1959
76 Laurell C B and Nyman M Studies on the Serum Haptoglobin Level in Hemoglobinemia and Its Influence on Renal Excretion of Hemoglobin Blood 12 493 1957
77 Lederer M Form of Acute Hemolytic Anemia—Probably of Infectious Origin Am J M Sc 170 500 1925
78 Lederer M Three Additional Cases of Acute Hemolytic Anemia Am J M Sc 179 228 1930
79 Leikin S L The Aplastic Crisis of Sickle Cell Disease Occurrence in Several Members of Families Within a Short Period of Time A M A J Dis Child 93 128 1957
80 Lerner A B and Watson C J Studies of Cryoglobulins Unusual Purpura Associated With Presence of High Concentration of Cryoglobulin (Cold Precipitable Serum Globulin) Am J M Sc 214 410 1947
81 Lerner A B Barnum C P and Watson C J Studies of Cryoglobulins Spontaneous Precipitation of Protein From Serum at 5 C in Various Disease States Am J M Sc 214 416 1947
82 Lipton E L Elliptocytosis With Hemolytic Anemia the Effects of Splenectomy Pediatrics 15 67 1955
83 McBryde R R Hewlett J S and Weisman R Jr Elliptocytosis a Study of Erythrocyte Survival Using Radioactive Chromium (Cr) Am J M Sc 232 259 1956
84 McDougal R A Shively J A and Palmer C Paroxysmal Nocturnal Hemoglobinuria in a Negro Child A M A J Dis Child 97 92 1959
85 McPhee W R Acquired Hemolytic Anemia Caused by Ingestion of Fava Beans Am J Clin Path 26 1287 1956
86 Macauley D Acholuric Jaundice in a Newborn Infant Arch Dis Childhood 26 241 1951
87 Martin W J and Heck F J The Porphyrins and Porphyria a Review of Eighty One *Cases Am J Med* 20 239 1956

CHAPTER 16

The Hereditary Hemoglobinopathies

The far reaching observations of Pauling and associates[134] which demonstrated that the sickling phenomenon was dependent upon an abnormal hemoglobin stimulated an intense interest in human hemoglobin in normal and disease states Rapidly accumulating information resulted in the identification of a large number of abnormal hemoglobins and showed that they were genetically controlled This added information documented the concept that the clinical and pathologic features of many of the hereditary hemolytic disorders are basically conditioned by chemical and physical changes in the hemoglobin[94,96]

The hemoglobinopathies comprise genetically determined disorders in which synthesis of normal adult hemoglobin is partially or completely suppressed causing the normal hemoglobin to be replaced by a hemoglobin variant including the fetal variety Thalassemia is conveniently grouped with the hemoglobinopathies because of the large amounts of fetal hemoglobin replacing the normal adult type in patients with severe disease and the combination of fetal hemoglobin with other well-defined abnormal hemoglobins No abnormal hemoglobin however has been described in patients with thalassemia

The description of specific hereditary hemoglobinopathies necessitates a preliminary consideration of methods by which the abnormal hemoglobins are identified and an analysis of their genetic transmission

Methods for Determining the Hemoglobin Types Differences between the hemoglobin of the human fetus and that of the adult have been known since 1866 when Korber[175] demonstrated that fetal hemoglobin was more resistant to denaturation by alkaline solutions than was the adult type Two simple procedures have served to identify the fetal and other types of hemoglobins Fetal hemoglobin is determined by the method of alkali denaturation and the abnormal hemoglobins have been separated by the technique of paper electrophoresis

Alkali Denaturation Method for Fetal Hemoglobin (Hemoglobin F) The fetal hemoglobin content is determined by the "one point" denaturation technique described by Singer and co-workers[144] With this technique an approximate 10 per cent hemoglobin solution is denatured for exactly one minute by $N/12$ NaOH the reaction being halted and the denatured hemoglobin precipitated by the addition of a 50 per cent saturated ammonium sulfate reagent. The hemoglobin

113 Stamey C C and Diamond L K Congenital Hemolytic Anemia in the Newborn Relationship to Kernicterus A M A J Dis Child 94 616 1957
114 Stats D Wasserman L R and Rosenthal N Hemolytic Anemia With Hemoglobinuria Am J Clin Path 18 757 1948
115 Suess J Limentani D Dameshek W and Dolloff M J A Quantitative Method for the Determination and Charting of the Erythrocyte Hypotonic Fragility Blood 3 1290 1948
116 Szeinberg A Asher Y and Sheba C Studies on Glutathione Stability in Erythrocytes of Cases With Past History of Favism or Sulfa Drug Induced Hemolysis Blood 13 348 1958
116a Tuttle A H Demonstration of Hemoglobin Reactive Substance in Human Serum Science 121 701 1955
117 van Loghem J J Jr Mendes de Leon D E Frenkel Tietz H and van der Hart M Two Different Serologic Mechanisms of Paroxysmal Cold Hemoglobinuria Illustrated by Three Cases Blood 7 1196 1952
118 Witebsky F Ehrlich's Side Chain Theory in the Light of Present Immunology Ann New York Acad Sc 59 168 1954
119 Wyandt H Bancroft P M and Winship T O Elliptic Erythrocytes in Man Arch Int Med 68 1043 1941
120 Young L E Hereditary Spherocytosis Am J Med 18 456 1955
121 Young L E Hemolytic Disorders Some Highlights of Twenty Years of Progress Ann Int Med 49 1073 1958
122 Young L E Izzo M J Altman K I and Swisher S N Studies on Spontaneous in Vitro Autohemolysis in Hemolytic Disorders Blood 11 977 1956
123 Young L E Izzo M J and Platzer R F Hereditary Spherocytosis Clinical Hematologic and Genetic Features in 28 Cases With Particular Reference to the Osmotic and Mechanical Fragility of Incubated Erythrocytes Blood 6 1073 1951
124 Young L E Miller G and Swisher S N Treatment of Hemolytic Disorders J Chron Dis 6 307 1957
125 Young L E Platzer R F Ervin D M and Izzo M J Hereditary Spherocytosis Observations on the Role of the Spleen Blood 6 1099 1951
126 Zetterstrom R and Strindberg B Sporadic Congenital Spherocytosis Associated With Congenital Hypoplastic Thrombocytopenia and Malformations Acta paediat 47 14 1958
127 Zinkham W H and Childs B Effect of Vitamin K and Naphthalene Metabolites on Glutathione Metabolism of Erythrocytes From Normal Newborns and Patients With Naphthalene Hemolytic Anemia A M A J Dis Child 94 420 1957
128 Zinkham W H and Childs B A Defect of Glutathione Metabolism in Erythrocytes From Patients With a Naphthalene Induced Hemolytic Anemia Pediatrics 22 461 1958
129 Zinkham W H and Diamond L K In Vitro Erythrophagocytosis in Acquired Hemolytic Anemia Blood 7 592 1952
130 Zinkham W H and Lenhard R E Jr Metabolic Abnormalities of Erythrocytes From Patients With Congenital Nonspherocytic Hemolytic Anemia J Pediat 55 319 1959

a solution of veronal buffer (pH 8.6 ionic strength of approximately 0.06 and a specified voltage and current flow) the various hemoglobins migrate toward the anode according to their individual mobilities. After an initial period of two to three hours the hemoglobins have separated sufficiently to be recognized the length of the complete run (usually four to six hours) depending upon the particular requirements of the laboratory. At the end of the separation the strips are removed and dried and amounts of each hemoglobin may be roughly estimated or accurately measured.

Designation of Hemoglobin Types. Recommended nomenclature designates the capital letter A for normal adult hemoglobin, F for fetal hemoglobin and S for sickle cell hemoglobin and consecutive letters of the alphabet for abnormal types in order of their identification. Case reports have established the importance of types C, D and E which occur in association with well defined hematologic entities. The racial incidence of hemoglobins is exemplified by the predominance of the abnormal hemoglobins S, C and D in Negroes and E in natives of Thailand.

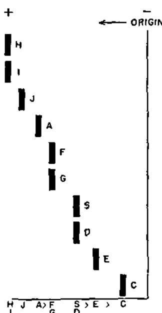


FIG. 12. Schematic representation of relative mobility of individual hemoglobins at pH 8.6. (From Smith, C. H.: The Abnormal Hemoglobins. Clinical and Hematologic Aspects. J. Pediat. 50:91, 1957.)

Electrophoretic Mobility of the Individual Hemoglobins. According to the specific conditions prescribed for paper electrophoresis of the common hemoglobin types, hemoglobins H and I move farthest toward the anode and C is the slowest remaining near the starting point. Intermediate between A and C in descending order of slower mobility are the hemoglobins F, G, S, D and E.

concentration in the filtrate (undenatured) is determined photoelectrically and the percentage of undenatured or alkali resistant hemoglobin (fetal hemoglobin) is calculated

Jonxis and Huisman using another alkali denaturation technique [?] in which readings are obtained from a curve constructed from sequential readings over a period of twelve minutes show a variance with the one minute denaturation method of Singer and co-workers The former showed that at high percentages of fetal hemoglobin the results obtained with the one minute method are about 10 per cent too low This is attributed to the fact that in completely denatured hemoglobin is removed by precipitation and filtration At concentrations below 10 per cent of fetal hemoglobin this method is less accurate and more difficult to interpret A combined alkaline denaturation and spectrographic method has al o been described suitable for the range of 1 to 10 per cent fetal hemoglobin and indicates its presence at the trace level (0 4 to 1 per cent)

Electrophoresis The migration of charged particles in an electrolyte solution which takes place when an electric current is passed through the solution is termed electrophoresis In this process particles bearing positive charges migrate to the cathode while negatively charged particles move to the anode This principle has provided a means by which plasma proteins and more recently hemoglobin solutions exposed to an electric field under selected conditions are separated in accordance with their isoelectric points and the individual mobilities of each constituent The speed and direction of forward migration depend upon the type of solution used to conduct the electric current its pH the ionic strength and the strength of the electric current

The moving boundary method of Tiselius previously extensively employed has been recently simplified with the use of paper electrophoresis With the latter a strip of filter paper properly prepared permits the differentiation of serum proteins and of hemoglobins according to their different velocities In contrast to the use of an acid pH (cacodylate buffer of pH 6 5) for the method of moving boundary electrophoresis the filter paper method of zone electrophoresis employs an alkaline buffer of pH 8 6 in which negatively charged protein particles move toward the positive electrode (the anode) With the contemporary interest in the relationship of abnormal hemoglobins to the hereditary hemolytic anemias paper electrophoresis has provided a readily accessible tool in the identification of the hemoglobins This method incorporates certain basic features which can be briefly outlined

PAPER METHOD In the paper method of electrophoresis the strip of paper is maintained in the horizontal position or is raised in the middle by suspension on one or more supporting rods In either case the paper is saturated with buffer solution with the ends dipping into containers of the same solution In the horizontal type of paper electrophoresis a length of filter paper wet with buffer solution rests on a plate of glass or plastic material with projecting points for support with the overlapping ends of the paper dipping vertically into troughs filled with the same solution In adjacent compartments at each end are placed the respective electrodes Before the solution is covered minute quantities of the red cell hemolysate to be tested (prepared from the hemolysis of red cells in distilled water) are applied to the filter paper close to the cathodal end In

the child and adult 2 per cent represents the upper limit of normal In certain of the chronic hereditary hemolytic disorders fetal hemoglobin reappears in substantial amounts Large quantities of fetal hemoglobin are synthesized in patients with sickle cell anemia (5 to 15 per cent) severe Cooley's anemia and to a lesser extent spherocytic anemia (usually less than 10 per cent)[184] The highest concentrations of alkali resistant hemoglobin are found in patients with severe Cooley's anemia in whom it may represent almost the entire hemoglobin content (12 to 100 per cent) The reason for these excessive amounts of embryonic hemoglobin is unknown and cannot be correlated with the severity of the disease Since multiple transfusions tend to depress hemoglobin production true estimates of its concentration can be made only during treatment free intervals or prior to the initiation of blood transfusions[175]

The presence of high concentrations of fetal hemoglobin in early infancy apparently suppresses formation of sickle hemoglobin[194] and hemoglobin C[189] Sickling gradually increases after birth to reach maximal levels at 4 months of age The replacement of fetal cells which are incapable of being sickled by red cells formed during the early months of life which are capable of being sickled is more closely correlated with the drop of fetal hemoglobin than with the absolute percentage of hemoglobin S[157] This state of high level fetal hemoglobin and minimal sickle cell formation may serve as a protection against the probable dire effects of intracapillary sickling in utero where low oxygen tension prevails

Fetal hemoglobin may be elevated in patients with pure red cell anemia (chronic congenital aregenerative anemia) acute and chronic leukemia and metastatic carcinoma involving the bone marrow Normal values are present in patients with iron deficiency anemia and acquired hemolytic anemia[184]

Primitive Hemoglobin (P) Fetal hemoglobin is to be differentiated from the primitive type (P) which is found in early fetal life This hemoglobin with an electrophoretic mobility on filter paper slower than those of the adult and fetal hemoglobins prevails during the first half of embryonic life and is later replaced by fetal hemoglobin The primitive hemoglobin is associated with severe hypoproteinemia and agammaglobulinemia but its biologic significance awaits further study[74]

Hereditary Aspects One of the first steps in the diagnosis and appraisal of the degree of involvement in a patient with suspected hereditary hemoglobinopathy is the search for specific hemoglobins in the affected person and in other members of the family

The principles relating to the familial distribution of the genes for the normal and abnormal hemoglobins conform to the basic principles of heredity In the somatic cells of the body the genes are paired one member of each pair being derived from one parent The members of a pair of genes are termed alleles and occupy similar positions or loci on a pair of homologous chromosomes The homozygous person receives an identical gene for the particular hemoglobin from both parents whereas the heterozygous person receives different contrasting or alternate members of genes or alleles In the homozygous state both genes of the pair are either normal or abnormal and in the heterozygous individual one of the genes is normal and the other abnormal In the case of abnormal hemo

Type I is imperfectly separated from type A unless F appears in great excess Hemoglobins S and D have identical mobilities In its homozygous form type G migrates at a single focus between type S and I and is differentiated from the latter by its lack of resistance to alkali denaturation[10] The mobilities of types H and I appearing closest to the anodal end of the filter paper are greater than type A The recently described hemoglobin J[1] possesses an electrophoretic mobility between hemoglobins I and A

The relative mobility rates are summarized in Fig 12 and are represented schematically in Fig 13

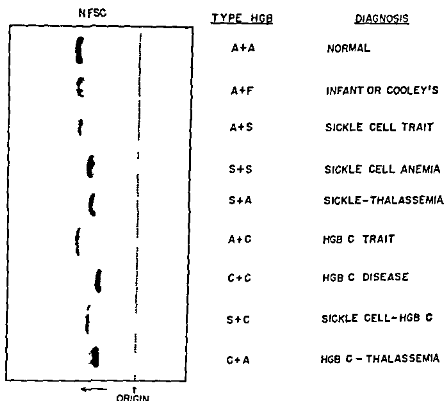


Fig 13 Electrophoretic patterns of the common hereditary hemoglobinopathies (From Smith C H The Abnormal Hemoglobins Clinical and Hematologic Aspects J Pediat 50 91 1957)

Fetal Hemoglobin Important biochemical differences exist between adult hemoglobin and the form elaborated by the human fetus during prenatal life Although the heme component of the two hemoglobin molecules is similar the structure and properties of globin and possibly of the heme globin linkages are different The property by which fetal hemoglobin is best known and most readily identified is its increased resistance to denaturation by alkaline solutions

Fetal hemoglobin which comprises 44 to 89 per cent of the hemoglobin in the newborn infant[143] decreases rapidly during the first year of life and is rarely demonstrable after the age of 30 months[3] Following this period and in

Prior to the discovery of abnormal hemoglobins it had been established that the gene responsible for Cooley's anemia or thalassemia occurs once in the heterozygous or mild disease and twice in the homozygous or severe disease. A similar pattern of inheritance was postulated for sickle cell disease[131]—namely that the gene responsible for sickling is present in the heterozygous sickle cell anemia. Exceptions to this genetic pattern—namely the failure to find similar recognizable blood changes in each parent of an affected child with either Cooley's anemia or sickle anemia—can now be explained by the presence of another unrelated abnormal hemoglobin in the dissimilar parent.

Target Cells. Target cells are particularly prominent in the blood smear of patients with abnormal hemoglobins and often provide a clue to diagnosis. In the Negro patient this distinctive feature is particularly suggestive of the presence of abnormal hemoglobins. Present in patients with both mild and severe Mediterranean anemia (usually up to 10 per cent), target cells are further increased in patients with sickle cell anemia and appear in even larger numbers in persons

Table 13. Common Abnormal Hemoglobin Syndromes

Type	*Diagnostic Features of the Blood Smear*	*Per Cent Abnormal Hemoglobin*
Sickle cell trait	Normocytic and normochromic red blood cells	24 to 45% Hb S
Sickle cell anemia	Normochromic or slightly hypochromic red blood cells, anisocytosis, poikilocytosis, polychromasia, target cells, sickle cells, occasional normoblasts	95 to 100% Hb S
Sickle cell–thalassemia disease	Large thin macrocytic cells, marked anisocytosis, basophilic stippling, nucleated red blood cells, moderate number of target cells, occasional sickled cells	67 to 82% Hb S
Homozygous hemoglobin C disease	Slightly microcytic or normocytic, normochromic red blood cells, large number of target cells	100% Hb C
Hemoglobin C trait	Normocytic, normochromic red blood cells, target cells may be absent or increased	28 to 44% Hb C
Sickle cell hemoglobin C disease	Normochromic, slightly microcytic red blood cells, minimal anisocytosis and poikilocytosis, numerous target cells	Hb S and Hb C each about 40 to 60%
Thalassemia hemoglobin C disease	Microcytic, minimally hypochromic red blood cells, marked anisocytosis and poikilocytosis with oval cells, basophilic stippling and microspherocytes	29 to 93% Hb C (usually 70 to 90%)

globins the double heterozygous state is one in which the genes determining the two hemoglobins differ from each other. In exceptional cases of thalassemia-sickle cell disease and of hemoglobin C-thalassemia, other patterns of genetic transmission than *multiple alleles* are to be considered. These rare instances involve such chromosomal relationships as linkage, in which genes are located in two different chromosomes, permitting independent transmission.

Comprehensive family pedigrees will frequently elucidate not only the hematologic picture of the patient but also that of other closely related persons who are asymptomatic or who have *suffered from a mild but unexplained anemia*. Numerous family studies have demonstrated that, with the exception of fetal hemoglobin, the genes responsible for the abnormal hemoglobins are alleles of those for normal hemoglobin.

The gene for any one of the abnormal hemoglobins may thus replace one or both genes for normal hemoglobin. When only one normal gene is replaced, the person is regarded as a carrier possessing a heterozygous hemoglobin trait. Homozygous or pure hemoglobin disease results when both genes for hemoglobin A are replaced by those of the same abnormal hemoglobin. With few exceptions, family studies have demonstrated that hemoglobins S, C, D, and E represent alleles of normal hemoglobin A.

Relation of Genetic Composition to Clinical Hematologic Variations. Expressivity of a gene refers to the variability of its expression in different persons and *explains certain of the quantitative aspects observed in the inheritance of the* abnormal hemoglobins. It has been postulated that the amount of a particular hemoglobin in a blood sample is influenced by interaction with the gene controlling another type of hemoglobin with which it is combined. Thus, in a combination of Cooley's anemia and sickle cell disease the gene for Cooley's anemia permits the appearance of amounts of sickle cell hemoglobin as high as 80 per cent in the offspring, despite the presence in the parent of less than 50 per cent of this component. This augmenting influence, which is also seen in *the interaction of the gene for Cooley's anemia with the genes for either hemoglobin* C and E, will be elaborated later.

The intensity of each syndrome varies from the asymptomatic or mild case in which a single abnormal hemoglobin is present in the heterozygous state in combination with one of the two allelic genes for normal hemoglobin, to the severe case in which two identical abnormal hemoglobins appear in the homozygous state.

Intermediate grades of hemolytic disease are associated with the presence of *two different abnormal hemoglobins—the mixed or double heterozygous* state. Their simultaneous presence results in a hemolytic anemia of variable intensity together with moderate splenomegaly and peripheral blood changes characteristic of both diseases. Although a single dose of a gene for abnormal hemoglobin produces the trait or mild disease and a double dose the severe disease, the degree of clinical and hematologic changes cannot be exactly correlated with one particular mixture of abnormal hemoglobins. Thus Cooley's anemia and sickle cell anemia, in both of which the homozygous state is represented, are more severe clinically and hematologically than either homozygous hemoglobin C or E disease.

in the hemoglobin. Pauling and associates[13] demonstrated that the electrophoretic mobility of sickle cell hemoglobin differed from normal hemoglobin. They also pointed out that these differences were based upon the number and kind of ionizable groups located in the globin fraction of hemoglobin rather than in the heme and that this molecular abnormality was responsible for the varied phenomena characterizing sickle cell anemia.

Hemoglobins are also identified by examining the digested fragments of the hemoglobin molecules on a chromatogram. Of the 300 amino acids forming the polypeptide chain of a half molecule of hemoglobin, a significant difference is observed in sickle and other hemoglobins as compared with normal hemoglobin. Ingram[89] found that normal and sickle hemoglobin differ in one portion of their polypeptide chains. In each sickle peptide having nine amino acids, valine replaces the normally occurring glutamic acid. This is presumably sufficient to alter the charge distribution on the surface of the molecule toward one favoring easy crystallization. Using the same method of trypsin and chymotrypsin digestion followed by "fingerprinting" of the peptide mixture, Ingram[93] also noted that the same glutamic acid of normal hemoglobin is replaced by the amino acid lysine in hemoglobin C disease. These findings with respect to both sickle cell hemoglobin and hemoglobin C reinforce the genetic evidence that these two mutations occur in similar places on the gene since they affect the same amino acid.[93] According to this concept, an altered gene is responsible for a corresponding alteration in the amino acid sequence of the protein with which it is identified.

The intrinsic features of the sickling mechanism becomes apparent when hemoglobin is reduced following exposure to low oxygen tensions or pH. The simplest method by which deoxygenation is accomplished is by placing a drop of blood under a sealed cover slip and observing the progressive increase in the number of sickle cells. Sickle hemoglobin is much less soluble than normal hemoglobin and even more so in the reduced state. Exposure to an environment of reduced oxygen tension causes sickle hemoglobin to become more viscous. This transformation is accompanied by intracellular crystallization[133] and tactoid formation.[6]

Concentrated stroma-free hemolysates prepared from the blood of patients with sickle cell anemia are extremely viscous when sickle hemoglobin is present in reduced states. Increases in viscosity of the whole blood accompany the multiplication of sickled forms and contribute to slowing of the blood flow. The tactoid form represents an altered and orderly alignment of the abnormal hemoglobin molecules in the anoxic state producing the sickle and crescent forms. The sickled erythrocyte has been described as a hemoglobin tactoid distorted by retained stroma.[illegible] Crystallization accounts for the high concentration of hemoglobin in sickle cells attaining a mean corpuscular hemoglobin concentration of 50 to 60 per cent in the crystal as compared with values of approximately 34 per cent in the normal cell.

The amount of gelling of reduced hemoglobin S involved in tactoid formation is influenced by the companion pigment particles which interact with it. Reduced hemoglobin must be sufficiently concentrated (above 10 per cent) for gelling and tactoid formation to occur. Actually, it has been shown that a minimum of 7 per cent intraerythrocytic concentration of hemoglobin S pigment is needed to elicit the sickling phenomenon.[illegible] With lesser amounts the carrier may show a negative sickling test but sickle hemoglobin may be demonstrable by electrophoresis. The presence of hemoglobin A in the heterozygous person with the sickle

with the genes for both Cooley's anemia and sickle cell anemia (microdrepanocytic or thalassemia sickle cell disease) than in the person with only one of these genes for abnormal hemoglobin In persons with sickle cell hemoglobin C disease they are present to the extent of 10 to 90 per cent of all red cells It has been suggested that large numbers of target cells in the blood smear indicate the presence of hemoglobin C Target cells are also increased in patients with hemoglobin E disease perhaps less than in those with hemoglobin C disease Few or none of these cells are noted in reported cases of sickle cell-hemoglobin D disease[14] or of hemoglobins G H I and J

Syndromes Associated With the Abnormal Hemoglobins Investigation of the physicochemical properties of the hemoglobin molecule has been particularly revealing in explaining the variability in the clinical hematologic and genetic aspects of sickle cell disease and Cooley's anemia These studies have demonstrated that each of these diseases exist not only as the classical homozygous forms with well known manifestations but also as variants The latter though less severe nevertheless simulate sickle cell disease and Cooley's anemia clinically and hematologically The differences can now be explained on the basis of the simultaneous presence of another hemoglobin Thus the majority of abnormal syndromes to be described are initially diagnosed as either thalassemia or sickle disease

The multiplicity of combinations of the abnormal hemoglobins in the heterozygous double heterozygous and homozygous states precludes a discussion confined exclusively to a single abnormal hemoglobin so that overlapping comments are inevitable

SICKLE CELL DISEASE

Sickle cell disease is a comprehensive term used to include all those hereditary disorders whose clinical hematologic and pathologic features are related to the presence of sickle hemoglobin (hemoglobin S) in the red cells Sickle cell disease is found primarily in Negroes Relatively few cases have been reported in members of the white race The majority of these cases occurred in persons of Italian Greek and Sicilian origin suggesting an admixture of Negro blood with members of the Mediterranean races[61]

The sickle cell trait (sicklemia) is present in those persons who are heterozygous for the sickling character and represents the combination of the sickle cell gene and the gene for normal hemoglobin In persons with sickle cell anemia the gene for sickling is present in the homozygous state and results from the inheritance of sickling genes from both parents The presence of a gene for another abnormal type of hemoglobin or the gene for thalassemia should be suspected in a child with sickle cell anemia when the blood of only one of the parents shows the sickle trait

Sickling Phenomenon The sickling abnormality is attributed to a mutant gene which is responsible for the synthesis of a type of hemoglobin different from normal hemoglobin The red cells that sickle are biconcave discs indistinguishable from the normal erythrocytes containing no sickle hemoglobin Although it had been shown previously[3] that the basic abnormality of the sickle red cell resided

Sickle Cell Trait

Incidence and Geographic Distribution The sickle cell trait representing the heterozygous state (hemoglobins A and S) occurs in approximately 7 to 9 per cent of American Negroes [1, 9] A small number about 1 in 40 of those with sickling show sickle cell anemia The highest frequency of the trait has been reported from East Africa where it varies from 30 to 45 per cent in some Bantu tribes The peak of the incidence falls somewhat toward the west coast of Africa averaging 20 to 25 per cent on the coast [19] The high incidence of the sickle cell trait in several of the aborigine tribes of Southern India has suggested that the sickle cell trait may have been carried from this area to Africa [11]

Clinical and Laboratory Features The trait or sicklemia can be demonstrated in vitro by the ability of the erythrocytes to sickle when oxygen tension is lowered This is accomplished by a variety of techniques such as sealing a drop of fresh blood under a cover slip with petrolatum or more rapidly by mixing the blood with a reducing agent such as 2 per cent sodium bisulfite Sickling is slower in *patients with the trait and the red cells assume a holly leaf appearance rather* than the typical elongated pointed and filamentous form observed in patients with sickle cell anemia The amount of sickle cell hemoglobin in the blood of persons with the trait as determined by electrophoresis varies from 24 to 45 per cent of the total hemoglobin [9] Target cells are present in small numbers but the red cells in stained blood smears are predominantly normal The red cells of healthy persons with the trait have a normal life span [19, 187]

Persons with the trait are not anemic show no physical abnormalities and are usually asymptomatic Splenic infarction resulting from vascular occlusion by sickled cells has been reported in patients with the sickle trait at moderate or high altitudes during flight [9, 30, 4, 1, 4] Incapability of concentrating urine normally [104] *gross hematuria* and retinal hemorrhage[81] have *also been described* occasionally in patients with the trait

Sickling and Malaria Since the observations by Beet [10] that the sickle cell trait might protect the bearer from the effects of malaria many studies have been carried out to test this hypothesis According to this concept the survival of persons with heterozygous sickle cell disease could offset the loss of persons with homozygous disease through malaria caused by *Plasmodium falciparum* but not other malarial parasites and thus maintain the high rates of sickling in malarious areas Apparently sickle hemoglobin is unsuitable for growth of *P falciparum* Thus the loss of the sickle cell gene by early death of patients with homozygous sickle cell anemia is counterbalanced by the resistance of persons with the sickle cell trait to become infected with malaria This concept of a selective or reproductive advantage for the person with heterozygous sickle cell disease over the normal person (balanced polymorphism) was also extended to thalassemia since many of the patients with this disease come from districts severely affected by malaria [15]

More extended experience however based upon surveys in areas in which malaria and sickle cell disease prevail including direct inoculation of *P falciparum* into persons with and without sickle cells has given conflicting evidence

cell trait decreases the amount of hemoglobin S required for gel formation and type C reduces this further Sickling therefore depends not only upon the presence of hemoglobin S but also upon interaction with other hemoglobins Type F hemoglobin present in the homozygous patient with sickle cell anemia exerts no significant influence on the gelling phenomenon[184]

The sickling of the erythrocytes in patients with homozygous sickle cell anemia (S S) and other forms of sickle cell disease (thalassemia sickle cell and sickle cell hemoglobin C disease) is rapid and assumes chiefly a filamentous form In persons with the sickle cell trait a holly leaf rather than the filamentous form predominates The spicules of the holly leaf form represent the points of the tactoids[1,17]

The sickle distortion is reversible so that the filamentous sickle cell which develops in an environment of lowered oxygen reverts to the normal discoid form when normal oxygen tension is restored Oxygenation causes the crystallized hemoglobin S to go into solution again Two types of sickle cells have been described those reverting to the previously normal shape on exposure to oxygen and a smaller number which remain irreversible[1,6] Sickle cells appearing on the blood smears of patients with sickle cell anemia are of the irreversible variety The blood smear of persons with the sickle trait is devoid of sickle cells

Pathogenesis The varied manifestations of sickle cell anemia result from the insolubility of reduced sickle hemoglobin and the formation of sickle cells which follows exposure to lowered oxygen tensions existing in the tissues Although persons with the sickle cell trait heretofore had been regarded as uniformly asymptomatic it is now known that occasionally they too are susceptible to the same difficulties as the homozygous patient in situations in which there is reduced oxygen tension such as occur in airplane flights[114]

The entanglement and enmeshing of rigid and inflexible sickle cells with one another increases the internal friction of the suspension resulting in an increased viscosity of the whole blood[5] The sickling and increased blood viscosity combine to produce capillary stasis the formation of masses and plugs of impacted red cells thrombi hemorrhage vascular occlusion infarction and ischemic necrosis Infection presumably increases plasma viscosity and results in an extreme degree of erythrostasis

The formation of sickle cells increases vulnerability to mechanical fragility especially as the hemoglobin is deoxygenated[8] The shortened life span of the cells during movement in the circulation and passage through organs results in hemolytic anemia Selective sequestration and stagnation of sickle cells in the spleen[1,7] of the patient with sickle cell anemia probably results in their local destruction and for those cells that escape increased intravascular hemolysis The cells from patients with the sickle cell trait and those from the patient with sickle cell anemia differ with regard to increased mechanical fragility Mechanical fragility of the sickle cells of the patient with the trait begins to be affected at a level of deoxygenation at which the cells of the patient with the anemia already show a maximal increase In general the in vitro behavior of sickle hemoglobin and sickle cells at reduced oxygen tensions are correlated with the severity of the clinical and hematologic features[1]

Sickle Cell Trait

Incidence and Geographic Distribution The sickle cell trait representing the heterozygous state (hemoglobins A and S) occurs in approximately 7 to 9 per cent of American Negroes [1, 2] A small number about 1 in 40 of those with sickling show sickle cell anemia The highest frequency of the trait has been reported from East Africa where it varies from 30 to 45 per cent in some Bantu tribes The peak of the incidence falls somewhat toward the west coast of Africa averaging 20 to 25 per cent on the coast [49] The high incidence of the sickle cell trait in several of the aborigine tribes of Southern India has suggested that the sickle cell trait may have been carried from this area to Africa [11]

Clinical and Laboratory Features The trait or sicklemia can be demonstrated in vitro by the ability of the erythrocytes to sickle when oxygen tension is lowered. This is accomplished by a variety of techniques such as sealing a drop of fresh blood under a cover slip with petrolatum or more rapidly by mixing the blood with a reducing agent such as 2 per cent sodium bisulfite Sickling is slower in patients with the trait, and the red cells assume a holly leaf appearance rather than the typical elongated, pointed and filamentous form observed in patients with sickle cell anemia The amount of sickle cell hemoglobin in the blood of persons with the trait, as determined by electrophoresis varies from 24 to 45 per cent of the total hemoglobin [illegible] Target cells are present in small numbers but the red cells in stained blood smears are predominantly normal The red cells of healthy persons with the trait have a normal life span [illegible]

Persons with the trait are not anemic show no physical abnormalities and are usually asymptomatic Splenic infarction resulting from vascular occlusion by sickled cells has been reported in patients with the sickle trait at moderate or high altitudes during flight [illegible] Incapability of concentrating urine normally [101] gross hematuria [illegible] and retinal hemorrhages [illegible] have also been described occasionally in patients with the trait.

Sickling and Malaria Since the observations by Beet [illegible] that the sickle cell trait might protect the bearer from the effects of malaria [illegible] studies have been carried out to test this hypothesis According to this concept the survival of persons with heterozygous sickle cell disease could offset the loss of persons with homozygous disease through malaria caused by *Plasmodium falciparum* but not other malarial parasites and thus maintain the high rates of sickling in [illegible] various areas Apparently sickle hemoglobin is more unsuitable for growth of *P falciparum* Thus the loss of the sickle cell gene [illegible] early death of persons with homozygous sickle cell anemia is counterbalanced by the resistance of persons with the sickle cell trait to become infected with malaria This concept of a selective or reproductive advantage for the person with heterozygous sickle cell disease over the normal person [illegible] extended to the [illegible] [illegible] of the [illegible] disease [illegible] affected [illegible]

M[illegible] experience however [illegible] areas [illegible] malaria and sickle cell disease prevail [illegible] of *P falciparum* [illegible] sickle cell [illegible]

as to this protective relationship[11] There is greater unanimity with respect to children—namely those who are heterozygous for the sickling gene (hemoglobin A S) have a greater chance of surviving to reproductive age than do normal children when both are exposed to malarial infection[111]

Although this subject is still controversial it appears that possession of the sickle trait affords some degree of protection against infection with *P falciparum* The infection is of shorter duration and the parasite count is lower than in persons who do not possess the sickle cell trait[9] Hence those who are heterozygous for the sickle cell gene appear to have a selective advantage in regions in which malaria is common

Sickle Cell (Drepanocytic) Anemia

Sickle cell anemia is a hereditary form of hemolytic disease occurring almost exclusively in Negroes and is due to the presence in the red cells of sickle hemoglobin the gene for which is present in the homozygous state Its incidence in American Negroes is 0 3 to 1 3 per cent[1 9] The disease is marked by anemia by painful and febrile episodes occasionally by aplastic crises and by a variety of manifestations due to distortion of the red cells The clinical syndrome was first described by Herrick in 1910[8] in a Negro from the West Indies with chronic anemia in whose blood smear elongated and sickled cells were present The bulk of hemoglobin (60 to 98 per cent) is of the S variety with the remainder consisting of fetal (F) hemoglobin (usually 2 to 24 per cent) Normal (A) hemoglobin is absent unless the patient has had a transfusion recently

Pathology The basic changes in the tissues and organs are mainly due to a combination of capillary stasis obstruction by elongated and pointed sickle cells thrombosis of small vessels increased destruction of red cells and hemosiderosis The bone marrow is hyperplastic from excessive erythrogenesis and blood stasis[44] Of the nucleated cells in the marrow normoblasts predominate but more primitive forms of the red cell series are also present Only occasionally are sickled nucleated erythrocytes observed[44] Leukocytosis is marked and megakaryocytes are increased Hyperplasia of the bone marrow is especially marked in children and accounts for the osteoporosis and subsequent expansion of the medullary spaces observed in the roentgenogram Fat embolism secondary to necrosis of the bone marrow has been described[193] Infarction of bone followed by embolization of bone marrow and fat to various organs is observed in patients with sickle cell–hemoglobin C disease as well as in those with sickle cell anemia[1] Focal infarction of the marrow contributes to the bone pain of a crisis Aseptic necrosis of the femoral head has been related to thrombosis of blood vessels supplying the affected area[9 18]

Hemosiderosis from increased hemolysis of the patient s cells and occasionally from transfusions is especially pronounced in the liver bone marrow lymph nodes spleen and kidney

The spleen undergoes a series of changes progressing from congestive enlargement to fibrotic atrophy[4] In younger people the spleen is slightly or moderately enlarged The splenic pulp is a deep red color due to intense congestion and engorgement with sickle cells the sinuses are compressed and infarcts are

common Thickening of the capsule and fibrous tissue organization subsequently follows in areas of organizing hemorrhage The siderofibrotic lesions in persons with sickle cell anemia resemble the siderotic nodules or "Gandy Gamna bodies" of the spleen which occur in persons with spherocytic anemia and portal hyper tension[4]

In the final stages of the disease the spleen is a shriveled and scarred mass embedded in adhesions reflecting the replacement of the pulp by fibrous tissue Atrophy from this sequence occasionally results in extremely small spleens (autosplenectomy) The size of the spleen therefore decreases as its vascularity diminishes as the hemorrhages and infarcts become organized and as fibrous tissue replaces the residual pulp

The liver is often markedly altered both in structure and in function Liver dysfunction and necrosis are brought about by severe impairment of hepatic flow due to the combined effects of anemia and capillary obstruction by masses of sickle cells and Kupffer cells distended with phagocytosed red cells Focal necrosis loss of liver cells scarring and cirrhosis characterize liver involvement[6] Cholelithiasis due to increased destruction of red cells is not infrequently observed in patients with sickle cell anemia[198]

The kidney lesions in patients with severe sickle cell anemia are marked by congestion of glomerular capillaries and of tubular arterioles with sickle cells and by hemosiderosis and scarring Hematuria is ascribed to congestion of small vessels thrombosis stasis and infarction necrosis[4] Changes in the central nervous system are primarily intravascular mainly due to thrombosis of small vessels in the meninges and cerebral cortex resulting in hemorrhagic degenerative and atrophic changes[8] Obliterative endarteritis of the cerebral vessels may also contribute to the causation of symptoms[13] Chronic leg ulcers occur in adolescents and adults frequently in the lower third of the leg on the inner side just above the ankle[118] They are usually punched out in appearance single or multiple and unilateral or bilateral The histologic picture is that of chronic granulomatous ulcer There is no evidence that ulceration originates from thromboses due to the blocking of capillaries by sickled erythrocytes[a]

Clinical Features Sickle cell anemia is usually recognized during the preschool period In one half of the patients symptoms are present by 2 years of age[1] although clinical manifestations are rare in the first six months of life In a few recorded cases the disease has been diagnosed between 1 and 3 months of age[3] In one series[1 3] the average age at onset of symptoms necessitating hospitalization ranged from 3 months to 15 years

As already pointed out excessive blood destruction and the pathologic tendency toward intravascular sickling stasis and thrombosis in various organ and tissue sites are responsible for the multiplicity of symptoms and the confusion with the other clinical syndromes In children the most frequent complaints are joint back and abdominal pain vomiting fever frequent upper respiratory infections fatigability and anorexia Other less commonly encountered symptoms are headache meningismus dyspnea weakness epistaxis (without purpura) convulsions lethargy and listlessness Episodes of acute abdominal pain may be severe accompanied by fever muscle spasm nausea vomiting

and leukocytes[118] These abdominal crises may have a fatal outcome and have been attributed to shock from massive sequestration of sickled red cells plasma los and circulatory failure[191]

Central nervous system manifestations may be the initial complaints or may occur later in the disease Pain in the extremities may be muscular or be based upon bone and joint involvement and often simulates osteomyelitis Arthralgia may be severe Inflamed and swollen joints occur but are infrequent Localized swellings presumably due to erythrostasis with sickle cells develop over bony structures such as the jaw and long bones Exacerbations of these complaints occur periodically and are often related to upper respiratory infections In infants under 2 years of age dactylitis with marked pain and swelling of the hands and feet are occasionally observed (hand foot syndrome) It has been stated that swelling of the hands and feet in a Negro infant in whom there is no readily apparent cause for the swelling warrants a suspicion of sickle cell anemia[1]

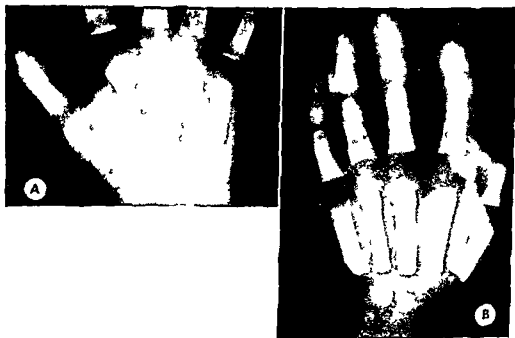


Fig 14 Hand foot syndrome in infants with sickle cell anemia A Note evid nce of sub periosteal bone formation and area of rarefaction in the body of the third metacarpal *B* Note complete regression *after one month these lesions are transitory*

Abnormal roentgenologic findings do not appear for the first week or ten days are confined largely to the metacarpals and metatarsals and extend infrequently to the phalanges Abnormalities *consist of blurring of the margins* and a variable degree of rarefaction within the medullary cavities Spontaneous regression occurs without residual signs[9, 19]

Physical findings vary considerably and include marked pallor of the mucous membranes greenish yellow *discoloration of the sclerae* lymphadenopathy car

diac enlargement and splenomegaly Liver enlargement may be slight to moderate The spleen often slightly to markedly enlarged in children atrophies with advancing age due to infarction and fibrosis The incidence of splenomegaly has been found to be 33 per cent in the first decade of life and 10 per cent thereafter [197] Although splenomegaly is not uncommon in patients with sickle cell anemia it appears to be much more frequent in those with sickle cell-hemoglobin C disease and sickle cell thalassemia disease An enlarged spleen is often associated with shortened survival of transfused normal cells [116] Less common are ascites joint swelling osteomyelitis gallstones and hemiplegia in patients with severe nervous system involvement The veins of the retina may be dilated and tortuous the arteries show this feature to a lesser extent [116a] Retinal hemorrhage has been observed [81] Enlargement of the heart and the occurrence of apical systolic murmurs are indicative of hypertrophy and dilatation in response to prolonged anemia and anoxia [146] Pericarditis is also noted Episodes of bronchopneumonia occur commonly and are attributable both to infection and to pulmonary infarction Cor pulmonale in older patients may be the consequence of multiple recurrent infarctions [1 2]

The inability to concentrate urine normally is a feature of sickle cell anemia and to a lesser extent the sickle cell trait [2 1 4 110] In patients with sickle cell anemia the concentration defect is corrected by transfusion of normal red cells which are known to suppress intravascular sickling [1 48 174] Children with sickle cell anemia are predisposed to salmonella osteomyelitis [8 9] Aseptic necrosis of the capital epiphysis of the femur and less commonly of the humerus occurs especially in older children and adults with homozygous sickle cell disease [70 16] sickle cell-hemoglobin C disease and sickle cell thalassemia The osseous lesions probably result from thromboses of the blood vessels supplying the affected areas presumably due to the sickling phenomenon Involvement of the femoral neck and head produces the clinical and pathologic picture of classical Legg Perthes disease Abnormal electroencephalograms are frequently observed in patients with sickle cell anemia and probably reflect blocking of the blood supply to various parts of the brain with varying damage to nerve cells [1] In contrast to its frequent presence in adults chronic leg ulceration is rare in children

The typical linear habitus with narrow hips and shoulders decreased stature increased upper dorsal kyphosis and lumbar lordosis increased anteroposterior diameter of the chest and hypogonadism are common in the adult patient Many of these changes are also present in children although to a lesser extent unless the disease is severe in early life Children often have a barrel shaped chest an enlarged and protruding abdomen and thin extremities [3]

Skeletal Changes The skeletal abnormalities as revealed by roentgenographic examination are similar to those seen in patients with severe Cooley s anemia but are less marked and less common [17] They are based upon bone marrow overgrowth resulting in osteoporosis widening of medullary spaces and thinning of the cortices These alterations are observed in the skull vertebrae long bones hands and feet The skull changes consist of widening of the diploic spaces external displacement of the outer table and a hair on-end appearance produced by the radial arrangement of the bony trabeculae so common in patients with

Cooley s anemia Skeletal changes are more frequent in adolescents and adults with sickle cell anemia than in children In adult life the hematopoietic tissue is replaced by connective and osteoid tissue New bone formation on the inner aspect of the cortex accounts for the thickening of the cortex and narrowing of the medullary cavity [17] Osteoporosis destruction and collapse of the weakened vertebral bodies especially in the lower thoracic and lumbar regions cause com pression deformities in both children and adults [50]

Blood The anemia is chronic normochromic and normocytic with signs of increased regenerative activity of the bone marrow The blood smear shows round and oval cells anisocytosis and poikilocytosis Hyperchromia and poly chromatophilia are less common Macrocytosis appears when the anemia is severe Sickled cells vary in number and are often surprisingly few in patients with severe disease One of the standard deoxygenation techniques shows sickling to be complete and rapid in all red cells in patients with sickle cell anemia in contrast with slow development in the red cells of those with the sickle cell trait Sickle cells (drepanocytes) are crescent or holly leaf shaped in appearance in persons with the sickle cell variants but in those with the homozygous disease they are characteristically elongated filamentous pointed at each end curved in the middle and contain a full complement of hemoglobin Target cells are increased in number Nucleated red cells chiefly normoblasts vary from a few to many especially in patients with pronounced anemia and occur in the presence of pneumonia or other severe infections Osmotic fragility is decreased mechan ical fragility increased [8] and red cells have a shortened survival time [19 16]

Bilirubin ranges from 1 to 3 mg per 100 ml with the major portion of the indirect type The number of reticulocytes is increased ranging from 5 to 25 per cent Excessive reticulocytosis indicates a superimposed acute infectious process or an immune hemolytic anemia In one 18 month old infant with a herpetic lesion an added acquired hemolytic anemia was evidenced by a hemo globin level of 4 6 gm per 100 ml reticulocytes of 78 per cent and a transient positive Coombs test [100]

Patients with sickle cell anemia maintain a relatively stable hemoglobin con centration with equilibrium between blood destruction and production estab lished between 6 and 9 gm per 100 ml without transfusions This stability is ex plained by the increased effective erythropoiesis in patients with this disease de spite increased rates of destruction By contrast in patient with thalassemia major there is less blood destruction but also less effective production—hence the need for multiple transfusions [4] The red cells range between 2 and 3 5 million per cubic millimeter With an infection or aplastic crisis the hemoglobin may drop below 5 gm per 100 ml and the red blood count below 2 million per cubic millimeter

The leukocytes range between 10 000 and 20 000 per cubic millimeter A count of 30 000 or more occurs in patients with complications such as pneumonia and are associated with a marked shift to the left of polymorphonuclear neutro phils and the appearance of myelocytes The platelet count is normal or slightly elevated The sedimentation rate[1] is usually slow even in the presence of marked anemia because of the abnormal shape of the sickle cells and poor rouleaux formation



Fig 15 *A* and *B* Stained blood smears of patient with homo ygous sickle cell ane ua Note elongated and narrow sickle cells with pointed end The nons ckled red cells vary in size and are round or oval *A* Courtesy Dr Ralph L Engle Jr New York N Y *B* courtesy Dr Ralph L Engle Jr New York N Y and Dr David Lawrenz Lakeville Conn

Crises The symptomatic or clinical aspects of the crises in patients with sickle cell anemia are discussed separately from the characteristics of the aplastic and hemolytic crises in these patients

Symptomatic or Clinical Crises With rare exceptions a crisis is characterized by fever and severe pain in the joints back and extremities usually without exacerbation of the anemia At times these episodes are accompanied by a slight rise in hemoglobin concentration The number of reticulocytes serum bilirubin concentration and excretion of urobiligen in the urine and stool are not increased

This is the common type of crisis in patients with sickle cell anemia and is designated as the clinical and symptomatic type[11] There is suggestive evidence that this type of crisis in childhood is associated with a sharp shrinkage of the plasma volume so that the peripheral hemoglobin concentration may actually be elevated Furthermore the anemic patient with this disease has a blood volume characteristically *far in excess of the normal in periods of freedom from disability*[4,173] As would be expected in restoring expanded plasma levels during treatment with whole blood plasma or dextran the hemoglobin level may actually drop Sudden death occurring in infants and children during a severe crisis in sickle cell anemia has been reported[96] The most striking necropsy finding in these patients was congestion of the internal organs with sickled erythrocytes

Aplastic and Hemolytic Crises The existence of a hemolytic crisis accompanied by a sudden destruction of blood and hyperfunctioning bone marrow in patients with sickle cell anemia has been challenged[11] (p 217) The aplastic or aregenerative crisis on the other hand is well documented It is characterized by a rapidly developing anemia due to the cessation of red cell production and the continued destruction of sickled cells in the circulation However hemolytic crises in patients with sickle cell anemia have occasionally been described in conjunction with an associated infection such as pneumonia or septicemia and are accompanied by severe pain in the back abdomen and extremities A significant drop in red cells and hemoglobin and an increase in both the indirect serum bilirubin and reticulocyte count accompany these symptoms[60]

Nutritional megaloblastic anemia has been reported in patients with sickle cell anemia and sickle cell–hemoglobin C disease as in patients with other congenital hemolytic syndromes[93] It has been attributed to an excessive need for folic acid vitamin B_{12} or both during periods of active growth and in the presence of pronounced erythropoiesis A reversal to normoblastic erythropoiesis follows specific therapy Transient episodes of megaloblastosis of the bone marrow occurred in a patient with sickle cell anemia complicated by an extracorpuscular hemolytic state[133] In this patient a relative deficiency of hematopoietic factors could have resulted from the severe hemolytic process

Diagnosis *The demonstration of the sickling phenomenon in vitro prevents* mistakes in diagnosis The basic pathologic process accounts for diverse symptom complexes which *simulate rheumatic fever osteomyelitis poliomyelitis* encephalitis[1,9] and other neurologic disorders and appendicitis and other acute surgical emergencies

Although sickle cell anemia mimics rheumatic fever especially when associated with joint pains fever and leukocytosis the coexistence of the two diseases is rare[139] Episodes in which the patient appears critically ill with abdominal pain nausea and vomiting may be severe and recurrent and extremely difficult to differentiate from a surgical condition requiring a laparotomy *The utmost* conservatism must be exercised in management when it is established that the patient has sickle cell anemia It is entirely possible however for the patient to suffer from the same surgical emergencies as persons without sickle cells The *combination* of severe bone pain and fever requires differentiation from osteomyelitis Except for the infrequent complications of salmonella osteomyelitis

and aseptic necrosis of the femoral and humeral capital epiphyses osseous involvement requiring treatment is uncommon

Treatment Since there is no specific therapy symptomatic and supportive measures are mainly relied upon [111] The treatment of the patient with the painful or clinical crisis in whom anemia is not a major factor demands the most serious consideration Bed rest aspirin codeine or other analgesic and sedative drugs usually suffice to control symptoms in the average case Oral hydration intravenous infusions of glucose or dilute electrolyte solutions increases blood volume and expedites mobilization of stagnant or trapped sickle cells [1 1] Antibiotics are prescribed if infection is present Hemodilution tends to overcome the shrinking of plasma volume with which the crisis is associated and to restore it to precritical levels The excessive plasma volume noted in patients who are not in crisis observed in a small number of cases [4 1 3] emphasizes the need for fluids Plasma dextran and other plasma extenders[60 9] are useful in achieving this aim

In contrast to patients with severe Cooley s anemia whose blood levels decline progressively unless supported by transfusions are patients with uncomplicated sickle cell anemia whose hemoglobin levels tend to stabilize between 6 and 9 gm per 100 ml without transfusions Transfusions of packed cells are given for correction of severe anemia accompanying infection for aplastic crisis and for relief of extreme pain when other measures fail Transfusions raise hemoglobin levels increase the oxygen capacity of blood and reduce the number and concentration of sickle cells both by dilution and by the depressing action of blood supplements on hematopoiesis [1 48 111 1 8]

Oxygen therapy is useful in combating severe anoxemia especially in patients with congestive heart failure but should only be employed for short periods of time Continuous treatment with oxygen depresses bone marrow and aggravates the anemia [113] A variety of agents each designed to relieve the painful crisis through individual mechanisms have been employed These include Priscoline [1 2] ACTH and cortisone [1] and intravenously administered sodium carbonate [0] More extended trial is required with each of these methods of treatment before a definite opinion can be given as to their merit Cobaltous chloride may produce initial satisfactory hematopoietic responses but these are not sustained The serious disadvantage of its use in addition to anorexia nausea and vomiting is the depression of thyroid function

Splenectomy in the treatment of patients with sickle cell anemia has had its advocates [1 9] but it does not occupy the therapeutic position that it does in patients with spherocytic anemia and to a lesser extent Cooley s anemia In one series of patients (Dickstein and Koop quoted by Margolies[118]) with marked to moderate enlargement of the spleen no major crises followed removal of the spleen Since an enlarged spleen constitutes the main site of sickling sequestration and destruction of the sickled cells [10] benefits from splenectomy should be substantial Furthermore removal of a large spleen increases the average erythrocyte survival time of the patient s red cells by one third (a half life of 3 7 days of Cr [1] presplenectomy as compared with a half life of 11 4 days postsplenectomy) [1 3] Nevertheless because of the natural tendency for the spleen to shrink in size and to atrophy its removal is not advocated as a routine measure It should

be reserved for the occasional patient with a very large spleen who requires excessive transfusions. In such patients normal red cells are presumably destroyed by the development of an extracorpuscular hemolytic defect.[110]

There is no contraindication to tonsillectomy when attacks of acute tonsillitis are frequent and associated with respiratory infections and a crisis. Preoperative transfusions and other safeguards are necessary.[89]

Prognosis. The prognosis is very unfavorable, with a fatal outcome in many patients in childhood or early adult life. Death results from overwhelming infection, congestive cardiac failure, cerebral vascular accidents and abdominal crises.[101] With the use of antibiotics, chemotherapy, transfusions and oxygen in selected patients, life expectancy can be prolonged. The outlook for the patient with the variants of sickle cell disease is more favorable than for those with homozygous sickle cell anemia.[36]

THALASSEMIA (COOLEY'S ANEMIA, MEDITERRANEAN ANEMIA, ERYTHROBLASTIC ANEMIA, HEREDITARY LEPTOCYTOSIS)

Thalassemia is a hereditary hemolytic disorder occurring predominantly in persons of Mediterranean origin and is characterized by abnormalities of red cell structure and hemoglobin synthesis resulting from a defect in erythropoiesis. The disorder ranges in severity from the asymptomatic trait to the severe form requiring frequent transfusions. In its severe form it combines the features of a refractory anemia with typical roentgenographic, clinical and hematologic features.

Historical. In 1925 and in 1927 Cooley and associates[1] drew attention to an anemia which had heretofore been regarded as belonging to the heterogeneous group of von Jaksch's anemia but which possessed such well defined features as to constitute a definite clinical entity. Outstanding features were its anemia, icterus, familial aspects, characteristic facies, skeletal changes, splenomegaly and the appearance of large numbers of circulating normoblasts in the peripheral blood. Because of the latter aspect the disease was initially termed erythroblastic anemia. From studies of the families of patients with the severe disease it soon became apparent that the siblings and parents possessed the features of the disease in mild form.

Nomenclature. The designation of thalassemia for Mediterranean or Cooley's anemia has shown increasing usage with the recognition of the abnormal hemoglobins. Although there may be some reservations with regard to its derivation,[5] it lends itself to the terminology indicating combination with various abnormal hemoglobins and to qualification as to the degree of severity.

Race and Incidence. The majority of patients have a Mediterranean ancestry, most commonly Italian, Syrian and Greek. In Italy the highest prevalence is in the lower Po valley in northeastern Italy around Ferrara[3] and in southern Italy. The Italian islands of Sicily and Sardinia, the island of Corsica and the Greek islands of Cyprus and Crete have large concentrations of affected persons, probably due to inbreeding.[3] The disease has been reported in a variety of non-Mediterranean races with wide geographic distribution. It has been found among the

Chinese (spreading from Canton and Hongkong) and other Oriental Asiatic Indians Egyptians Britains Germans Bukharan and Kurdistan Jews[110] Negroes[8] and Mexicans to mention only a few[10] In some areas of Thailand[1 1] the incidence is high and represents one of the most common forms of hemolytic anemia Its spread from areas adjoining the Mediterranean Sea by Greek traders in commerce colonizations and explorations may account for the present day frequency with which this disease is found in southern Asia[18] It would seem that mass migrations carried the genetic defect eastward to China from a single focus in the northern Mediterranean[6] There is still the possibility however that spontaneous mutations arising in a number of areas account for the widespread nature of the thalassemia gene If the frequency with which the mild type of Cooleys anemia is encountered in New York City is an indication of its extent among persons of Mediterranean origin in other parts of the United States this condition is extremely prevalent In New York City persons with the mild or severe disease are already separated by several generations from those originally born in southern Italy and Greece Neel and Valentine[13] on the basis of known incidence of the severe form computed the mild form as occurring in approximately 4 per cent of adult Italians in the city of Rochester N Y The bulk of Italians immigrated to the United States from the southern part of their country in the latter half of the nineteenth century and the beginning of the twentieth[6] It is in the offspring of this group in New York City that the largest number of cases of Cooleys anemia occur

Genetic Transmission The person with the trait or mild disease who functions as a carrier is heterozygous for a gene which when homozygous produces the severe disease The gene for thalassemia has a varying expressivity for the grade of anemia and sometimes it is difficult to separate the moderate from the severe type hematologically The presence of the trait in only one parent of a child with the severe disease should prompt a search for the sickle cell trait hemoglobin C disease or hemoglobin E disease in the other parent and for the combined disease with the different hemoglobins in the patient

Effect of the Gene for Thalassemia Upon Other Hemoglobins The gene for thalassemia either interferes with the normal suppression of fetal hemoglobin or the latter is formed in place of the more difficult synthesis of the adult type of pigment The presence of excessive amounts of hemoglobin S C and E in combination with thalassemia may reflect a compensatory replacement of normal *hemoglobin whose synthesis is retarded by the gene for thalassemia* As already has been postulated these increased concentrations may also reflect the potentiating effect of the gene for thalassemia on the formation of abnormal hemoglobin Exception to this principle has been found in connection with a thalassemia gene associated with a low A hemoglobin percentage which fails to interact with the genes for hemoglobin S C and E This contrasts with the more common interacting thalassemia gene which is responsible for an elevated A component[8] (p 276)

Clinical Types The disease presents a wide and continuous spectrum of severity designated at each extreme as thalassemia major for the severe type and thalassemia minor for the mild type In the affected person these correspond to

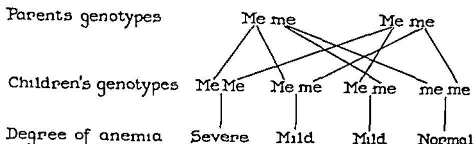


Fig 16

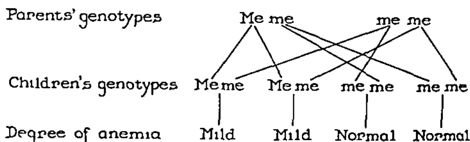


Fig 17

Figs 16 and 17 Schematic representation of genetic transmission in Cooley's anemia *Me is a gene for Mediterranean anemia (Cooley's anemia thalassemia)* me is the contrasting normal allele The designation of Me me is the heterozygote a simplification of current terminology for the trait or mild form of this disease Me Me is the homozygote (severe disease thalassemia major) me me is the normal individual

Fig 16 Inheritance of the severe type of Mediterranean anemia when both parents are heterozygous (From Smith C H The Abnormal Hemoglobins Clinical and Hematologic Aspects J Pediat 50 91 1957)

Fig 17 Inheritance of the mild type of Mediterranean anemia when one parent is heterozygous and the other normal (From Smith C H The Abnormal Hemoglobins Clinical and Hematologic Aspects J Pediat 50 91 1957)

the homozygous and heterozygous states respectively In both the major and minor types there are also varying grades of severity Thus thalassemia minor embraces forms which are asymptomatic without anemia (termed the trait or thalassemia minima) and the minor type per se which is usually associated with *mild to moderate anemia Patients with thalassemia minor or minima* being heterozygotes remain true to type and never develop the severe disease Italian writers refer to Rietti Greppi Micheli s disease as a heterozygous type much less severe than thalassemia major and described by them *as hemolytic icterus with increased* red cell osmotic resistance to fragility [1, 8]

The homozygous disease in which each parent is heterozygous for thalassemia falls into two classifications on the basis of grade of severity—the *intermediate* (moderate) type in which the patients function effectively without transfusions and the severe type in which the patients require multiple blood supplements to maintain hemoglobin levels compatible with normal activity In contrast to

the severely affected patients those in the intermediate group are able to maintain concentrations of hemoglobin ranging from 7 to 10 gm per 100 ml without treatment On the other hand patients with the intermediate type are observed in whom the disease is quiescent for many years until quite suddenly transfusions are required This occurs following infections or exposure to noxious agents or for no apparent reason From this point on the need for transfusions may become so urgent that splenectomy is necessary

Fetal Hemoglobin in Patients With Thalassemia In patients with the severe disease an extraordinary percentage of the circulating hemoglobin is of the alkali resistant or fetal type with values ranging from 40 to 100 per cent [11 144 06] In parents and asymptomatic siblings with the trait of the disease fetal hemoglobin lies within the normal range (less than 2 per cent)

Except for the marked elevation of fetal hemoglobin no specific abnormal hemoglobin has been described in patients with this disease No relationship has been demonstrated between the amount of fetal hemoglobin in an individual patient and the severity of the disease It has been suggested that the increase in fetal hemoglobin stems from interference by the gene for thalassemia with the synthesis of adult hemoglobin so that reversion to fetal hemoglobin formation results In several infants 1 and 2 months of age in whom clinical and hematologic features were already indicative of thalassemia major fetal hemoglobin exceeded the elevated levels normally found at this age [3] These observations indicate that the high level of fetal hemoglobin synthesis is a continuous process from fetal to adult life and that interference with the formation of normal adult hemoglobin is a permanent feature of the severe disease

With overnight electrophoresis fetal hemoglobin when present in substantial amounts can be demonstrated as a slower component than normal hemoglobin and almost continuous with it

Pathogenesis Defective synthesis of normal hemoglobin with an increased percentage of fetal hemoglobin impaired fabrication of circulating erythrocytes [194] and increased hemolysis of the defective cells have been implicated in the causation of the disease More specifically there is evidence that heme synthesis may be impaired by a relatively slow rate of protoporphyrin synthesis and by a partial block in the combination of protoporphyrin and iron Globin formation remains unaltered It has been demonstrated in patients with severe Cooley's anemia that the problem of establishing equilibrium compatible with normal activity is greatly exaggerated by the inability of erythropoiesis to compensate for the accelerated rate of red cell destruction and this inadequacy is greater than that in patients with other hemolytic anemias This accounts for the greater need for transfusion in patients with thalassemia than in those with sickle cell anemia in whom production is more effective

Pathology The anatomic changes reflect the abnormalities that characterize the disease—namely increased destruction of structurally defective red cells increased regeneration with erythropoietic hyperplasia of the bone marrow extramedullary hematopoiesis deposition of excessive iron in tissues resulting from hemolysis of transfused red cells and the patient's own red cells and the long term effect of chronic hypoxia

The skeletal structures contain abundant brown red and chocolate colored marrow the bony trabeculae are thinned and often the site of new bone formation The spleen is firm hard and greatly enlarged The pulp is abundant cellular and fibrotic tissue is increased Extensive hematopoiesis is noted chiefly of the red cell series and to a lesser extent of other myeloid elements The follicles are atrophied[9]

In some patients visceral siderosis and fibrosis of the liver and pancreas are especially marked The liver parenchyma is divided by broad irregular bands of fibrous tissue into nodules of varying sizes Extreme hemosiderosis is diffuse throughout the liver so that iron pigment often fills the cytoplasm of the cells Hepatic cells regenerate and show much less pigmentation In the pancreas extensive and diffuse interlobular and intralobular fibrosis is frequently observed with a similar division of the parenchyma into nodules The anatomic changes in the liver and pancreas are frequently identical with those characterizing idiopathic hemochromatosis[1] Lymph nodes show pigmentation and are also the site of increased erythropoiesis

Hemosiderin deposits are especially marked in the liver spleen and pancreas and are also found in the thyroid parathyroid and adrenal glands distal convoluted tubules of the kidneys bronchial glands gonads lymph nodes lungs and myocardium[7,11] The heart is dilated and hypertrophied showing dense hemosiderotic staining of the myocardium interstitial fibrosis and hypertrophy of the myocardial fibers

Clinical Features The clinical features of thalassemia major and thalassemia minor are discussed separately

Thalassemia Major Thalassemia major begins insidiously in infancy and is sufficiently developed in most patients to be recognized clinically in the latter half of the first year of life Pallor diarrhea fever poor feeding and an enlarged spleen in children of Mediterranean origin lead to suspicions of the diagnosis The severely anemic patient presents a pathognomonic facial appearance including prominent frontal and parietal bosses enlargement of the head prominent malar eminences depression of the bridge of the nose puffy eyelids a mongoloid slant of the eyes often with an epicanthal fold enlargement of the superior maxilla exposing the upper teeth a muddy yellow complexion and icteric tint to the conjunctivae Other characteristic features are small stature and hypogonadism Hypertrophy of the upper maxilla may lead to eventual marked malocclusion especially involving the frontal central incisors[19] (See Figs 18 and 19)

Lymphadenopathy is occasionally noted The abdomen protrudes when splenomegaly becomes prominent especially in the younger child Splenomegaly is progressive and abdominal enlargement becomes disabling Initially the liver is enlarged to a lesser degree but becomes markedly enlarged especially after splenectomy Gallstones[1,6] and chronic leg ulcers[31,39,134] are rare occurrences nosebleeds are more frequent Pathologic fracture of the femur is an infrequent complication and is due to cortical atrophy With prolonged and intensive transfusion therapy the clinical evidence of hemosiderosis is superimposed on the manifestations of chronic anemia The patient with Cooley s anemia who has had fre



Fig 18 Characteristic facies in severe Cooley's anemia (thalassemia major) Note prominent malar eminences depression of the bridge of the nose slight oblique appearance of eyes and enlargement of superior maxilla with protrusion of lip upward exposing upper teeth

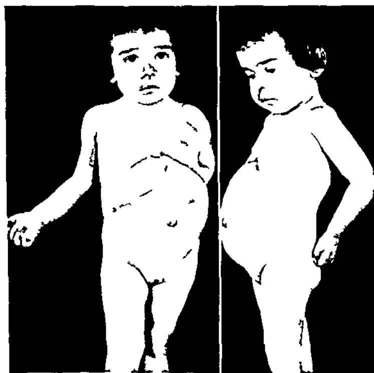


Fig 19 Severe Cooley's anemia showing enlargement of liver and spleen and protrusion of abdomen Facies are similar to those of patient shown in Fig 18

quent transfusions shows a deep brown pigmentation and darkening of the skin due to increased amounts of melanin in the epidermis and hemosiderin in the dermis [113] The skin gives a tanned bronzed coloration with fine dark stippled like freckles

Cardiac dilatation and enlargement and hemic murmurs are common Asymptomatic cardiac enlargement begins in late childhood and slowly increases Pericarditis of the acute benign nonspecific type has been described in patients with severe Cooley s anemia especially those who have had splenectomy [1] In all of *these patients the pericarditis was self limited* and ended in complete recovery The basis for this peculiar susceptibility is unknown Children with Cooley s anemia whose hemoglobin levels are markedly lowered undergo prolonged effort with surprisingly little disability Headache precordial and bone pain listlessness and anorexia appear when the hemoglobin levels drop below 7 gm per *100 ml* and are relieved by transfusions

Thalassemia Minor The patient with the trait is asymptomatic and the disease goes unnoticed usually being detected in the course of a family survey when a *sibling is suspected of having the severe disease Persons with the trait or mild* type do not show the cardiac murmur enlargement of the heart or bone and joint pain of the severe type Patients with mild or moderate anemia are often treated for iron deficiency anemia until the lack of response to therapy prompts a *blood examination and the true diagnosis is disclosed Those with the mild* disease show no facial abnormalities or skeletal changes In persons with the moderate or mild disease the spleen may be slightly enlarged or not enlarged at all

Growth and Maturation *In patients with thalassemia minor* growth is normal In some patients growth and maturation of the skeleton are retarded and Caffey[18] regards patients with Cooley s anemia as the best examples of skeletal dwarfism and infantilism caused by chronic anemia Children with the severe disease grow normally until about the age of 8 to 10 years at which time their growth rate undergoes marked retardation so that they attain a very short final height Secondary sexual characteristics develop later than in the normal population Normal menses are rare and cease several months to several years after the onset

Skeletal Changes The skeletal changes as revealed by roentgenographic examination in persons with Cooley s anemia reflect the overactivity and overgrowth of the bone marrow [1] Extreme marrow hyperplasia results in osteoporosis widening of the medullary spaces thinning of the cortex trabecular atrophy coarse reticulation with regeneration of new bone which is a later development in the disease and thickening of the skull The frontal bone is the site of early and marked thickening Overgrowth of the marrow in the paranasal sinuses and mastoids interferes with their pneumatization and occasionally completely suppresses it [18] Lateral *views of the skull show* an enlarged *diploic space which is* finely granular mottled or striated Appearing between the tables of the skull the perpendicular striations which seem to extend beyond the outer atrophied table give the appearance of hair standing on end The earliest skeletal changes are observed in the *small bones* particularly in the metacarpals and metatarsals

revealing osteoporosis and expansion of the medullary cavities producing a rectangular rather than a normal concave appearance Cortical thinning may be so extreme as to result in pathologic fractures

The character and degree of the bone changes are modified significantly with



Fig 20 Roentgenograms of a severe case of Cooley's anemia (thalassemia major) *A* Skull showing enlarged diploic space which is fairly granular mottled or striated note hair-on-end appearance Marked rarefaction of hand and wrists *B* and of lower extremities *C* *D* Note compression of vertebral bodies

age In older children the bone lesions regress in the more distal portions of the skeleton (hands arms and legs) whereas with advancing age red marrow is replaced normally with fatty marrow The characteristic changes described in the hands and other peripheral areas are thus diminished and may disappear at puberty [16] On the contrary in the skull spine and pelvis which are sites of active and persistent red marrow formation the roentgenographic changes become more conspicuous [16 18] Compression of the vertebrae quite common in patients with sickle cell anemia is rare in those with Cooley s anemia Severe reticulation and rarefaction of the vertebral bodies are not uncommon Localized tumors of red marrow in the generally hyperplastic marrow have been described Costal osteomas giving the appearance of large bony masses in the thorax without external signs of tumor have been described [1 3] In one of the patients the mass proved to be a large shell of bone containing red marrow and blood [18] In the patient with the trait or mild form of the disease skeletal changes are absent

Blood Picture The blood picture in patients with thalassemia major and in those with thalassemia minor provides important clues to the diagnosis of these diseases

Thalassemia Major The anemia is pronounced and of a hypochromic microcytic type similar to that seen in patients with severe iron deficiency The hemoglobin ranges between 5 and 9 gm per 100 ml and the red cell count between 2.5 and 3 5 million per cubic millimeter These values are obtained soon after diagnosis before any transfusions have been given and in those patients under treatment after a prolonged interval following transfusion so there is no admixture with donor blood In patients with the intermediate homozygous type the hemoglobin stabilizes between 7 and 10 gm per 100 ml without the need for transfusions The reduction in hemoglobin level and hematocrit is proportionally greater than the reduction in the number of red cells as occurs in patient with iron deficiency The erythrocytes show polychromatophilia marked hypochromia anisocytosis and poikilocytosis and are often fragmented

Although microcytes predominate the most important cells for diagnostic purposes are the large pale erythrocytes interspersed among them which contain irregularly distributed clumps of hemoglobin with the intervening areas apparently possessing staining defects These cells are extremely thin and leaflike and in wet preparations their edges fold over and the several layers thus formed possess a remarkable transparency In the stained smear the combination of scattered hemoglobin and the thinness of the cells result in bizarre and wrinkled shapes The abnormal thinness of the red cells in this disease has led to its designation as hereditary leptocytosis

A nonspecific macrocytic cell commonly found both in patients with the severe form and in those with mild forms of thalassemia is the target cell This erythrocyte has been so named because of its deeply stained center and periphery which are arranged in concentric light and dark zones Another macrocyte is a round or sometimes oval cell with a narrow rim of hemoglobin of varying thickness with a large zone of central achromia in which only a faintly stained island of hemoglobin is occasionally noted

Nucleated red cells are almost invariably found and constitute one of the most characteristic findings of the disease The size and maturity of these cells vary but most often they are typical normoblasts and mature micronormoblasts with pyknotic nuclei Normoblasts vary from a few to large numbers sometimes equal

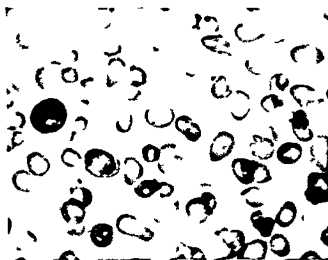


Fig. 21 Photomicrograph of blood smear of patient with severe Cooley's anemia (thalassemia major) presplenectomy Note microcytes hypochromic macrocytes anisocytosis and poikilocytosis Many of the red cells appear fragmented.



Fig. 22. Blood smear of patient with severe Cooley's anemia, postsplenectomy Note excessive size and abnormal thinness of red cells marked hypochromia, and large number of normoblasts

ing or exceeding the leukocytes numerically Erythroblastosis is extremely marked after splenectomy and constitutes a distinctive postoperative feature

Red cells containing Howell Jolly bodies are infrequently observed in patients with an intact spleen Polychromasia and basophilic stippling are present but are less marked in patients with the severe than in those with the mild type of Cooley s anemia because of the lack of hemoglobin Reticulocytes are increased and range from 2 to 8 per cent [1 1] Moderate leukocytosis is present with white cell counts ranging from 15 000 to 30 000 per cubic millimeter but the upper level may be exceeded Granulocytes predominate and myelocytes are common The platelet count is generally normal Moderate leukopenia and to a lesser extent thrombocytopenia are occasionally observed and attributed to hypersplenism[1 0] or suppression by multiple transfusions The bone marrow in severe cases shows primitive erythroid cells and a predominance of basophilic normoblasts microblasts and pronormoblasts

Aplastic crises have been described comparable to those in patients with spherocytic anemia and other hemolytic anemias resulting in erythroid marrow hypoplasia and reticulocytopenia [96]

The osmotic fragility is strikingly abnormal The red cells are markedly resistant to hemolysis in hypotonic sodium chloride solution so that in some instances *they are not entirely hemolyzed even in distilled water The thinness of the red* cells renders them capable of absorbing more water than normal before disruption The serum bilirubin is usually slightly elevated ranging from 1 5 to 3 mg per *100 ml When the upper limit is exceeded hepatitis cholelithiasis or a hemolytic* crisis should be considered The serum iron is increased and the iron binding protein is fully saturated [1 4]

Thalassemia Minor The essential features consist of hypochromic microcytes and macrocytes basophilic stippling oval and target cells and less frequently polycythemia[1 0 1] with or without slight anemia Osmotic fragility is greatly decreased *even in the absence of anemia*

The occurrence of morphologic changes of the red cells far in excess of anemia constitutes a fundamental principle in the detection of thalassemia minor The hemoglobin *level* in patients with *thalassemia* minor *is above 9 gm per 100 ml* Since the mean corpuscular volume of red cells (MCV) represents the ratio between the volume of packed cells and the erythrocyte count the smaller calculated value results from the increased numbers of *red cells per cubic millimeter* in relation to the normal or slightly lowered volume of packed red cells Hypochromic and microcytic red cells predominate simulating the blood smear of patients with iron deficiency anemia Varying *degrees of poikilocytosis* and *anisocytosis* are seen but the distinguishing feature is the interspersal of hypochromic macrocytes giving the smear a macrocytic appearance in many cases Target cells appear in fewer than half *of the patients oval cells* and *cylindrical or cigarette* shaped forms are common For unexplained reasons patients with the trait or mild anemia frequently show slight eosinophilia

Excessive numbers *of erythrocytes* with *punctate* basophilia are sometimes sufficiently pronounced to suggest lead poisoning Occasionally they are equally common in the sibling and one of the parents Elevated red cell counts

exceeding 6 million per cubic millimeter are occasionally noted in children but are most common in adults and are regarded as a compensatory physiologic response Normoblasts and myelocytes are not present reticulocytes seldom exceed 5 per cent We have seen several children with thalassemia minor whose hemoglobin during childhood ranged between 9 and 10 gm per 100 ml and showed a spontaneous rise to 12 and 13 gm during adolescence Occasionally



Fig 23 Photomicrograph (×900) from blood smear of patient with the mild type of Cooleys anemia (thalassemia minor) Note anisocytosis poikilocytosis hypochromic macrocytes microcytes and oval elongated and rodlike cell (From Smith C H Detection of Mild Types of Mediterranean (Cooleys) Anemia Am J D Child 75 505 1948)

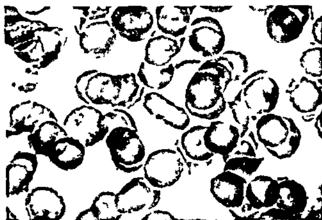


Fig 24 Photomicrograph (×900) from patient with mild type of Cooleys anemia Smear slightly overstained to demonstrate stippling

this change was accompanied by an elevated red blood cell count The blood smear however still demonstrated the typical morphologic features of thalassemia These changes are explained on the basis of the marked elevations of hemoglobin and red cells at times of a polycythemic level which occasionally appear in the normal adolescent

Erythropoietic hyperplasia of the bone marrow is less marked than in patients with thalassemia major Polychromatic and pyknotic normoblasts are common[7] The serum iron and latent iron binding capacity are normal Fetal hemoglobin levels are normal

Hemosiderosis and Hemochromatosis Any disorder which depends upon transfusions as the mainstay of treatment is subject to increased iron deposition In common with other refractory and chronic hemolytic anemias iron stores accumulate from both the degradation of hemoglobin (250 mg in each 500 ml of blood) and increased iron absorption from the diet by the gastrointestinal tract Another significant source is from the accelerated destruction of the patient s own defective red cells In patients with severe Cooley s anemia widespread visceral hemosiderosis and fibrosis frequently coexist although there is no uniformity about the progression from the former to the latter [1] It has been suggested that the development of true hepatic cirrhosis and fibrosis of the pancreas which characterizes hemochromatosis depends upon the intervention of accessory factors such as combined hypoxia in addition to large iron deposits[8] Both conditions prevail in patients with severe Cooley s anemia—hence the concern about the development of hemochromatosis With the advancing age of the child and progressive iron deposition patients will occasionally manifest diabetes and other clinical and pathologic features identical with those of endogenous hemochromatosis One of the serious complications of Cooley s anemia is irreversible heart failure in which myocardial siderosis plays a major etiologic role

Red Cell Survival The red cells of the patient with the severe disease transfused into a normal recipient show a half life of about twenty five days as compared with the mean half life of about sixty two days attained with normal erythrocytes[10] The shortened survival is also noted by the injection of the patient s own red cells labeled with Cr^{51} into his own circulation By this method about 20 per cent of the red cells have a life span of only several days and the remainder of about thirty days The survival time of erythrocytes from patients with the trait transfused into normal recipients falls within the normal range[11]

The use of Cr^{51} in determining the longevity of red cells is not impaired by the fact that accelerated rates of Cr^{51} elution take place from hemoglobin solutions containing excessive amounts of fetal hemoglobin such as occur in cord blood and in the blood of patients with severe Cooley s anemia This does not take place however from *intact red cells* containing large amounts of fetal hemoglobin[60]

To what extent the neutral mucopolysaccharide found in red cells of patients with severe Cooley s anemia contributes to their shortened life span is unknown

Diagnosis Thalassemia major possesses so many distinctive features that the diagnosis is rarely overlooked The association of the characteristic facies skeletal

changes massive splenomegaly severe anemia markedly hypochromic and distorted red cells stunted growth and usually Mediterranean parentage separates this disease from other hemolytic anemias Osmotic fragility is decreased and sickling and spherocytosis are absent The diagnosis is confirmed by examination of the blood of the patient s parents which shows decreased osmotic fragility basophilic stippling hypochromic microcytes and macrocytes and oval cells The child who shows only moderate anemia with many of the clinical features of the severe disease often represents a combination of both sickle cell disease and thalassemia The marked normoblastemia noted in the blood of patients with severe Cooley s anemia has led to confusion with di Guglielmo s disease a rare entity in childhood The occurrence of this erythroid proliferative disorder in a child with thalassemia has been described [134]

Thalassemia minor resembles iron deficiency so closely that the two are usually indistinguishable The blood smears are identical and show large numbers of hypochromic microcytes moderate stippling and oval and target forms The diagnosis is made by elimination since there are no pathognomonic features as are observed in patients with thalassemia major Frequently a diagnosis of thalassemia minor is suggested when a patient believed to have iron deficiency anemia has not responded to iron therapy The discovery in at least one parent of a blood picture similar to that of the patient an abnormal percentage of A hemoglobin and a normal serum iron content as contrasted with a low serum iron content in patients with iron-deficiency anemia establish the diagnosis of thalassemia minor In the adult patient thalassemia minor may coexist with pernicious anemia[34] or megaloblastic states [6]

Starch Block Electrophoresis of Hemoglobin in the Diagnosis of the Thalassemia Trait When hemoglobin analyses are performed by paper electrophoresis at a pH of 8 6 two subtypes of normal hemoglobin are found In addition to the main component A_1 slow and fast components appear which are designated as A and A_3 respectively By the Tiselius moving boundary method performed at a pH of 6 5 A in contrast to its reaction during paper electrophoresis migrates faster than A_1 [166]

An alternate method in which a starch block prepared from potato starch powder is substituted and hemolysates prepared in an identical manner as in the paper method are used provides electrophoretic patterns of diagnostic value in patients with thalassemia [64 109 166]

The starch block suspended in an arbitrarily set volume of barbital buffer at a pH of 8 6 (ionic strength 0 05) is covered with Pliofilm and transferred to a cold room where electrophoresis is carried out at 4 C at a specified voltage and current flow Three major hemoglobin fractions can be readily observed A_1 A_2 and A in order of their speed of migration toward the anode The A component resembles hemoglobin E in electrophoretic mobility The percentage of each component of A is obtained by elution from the respective areas of the block and determined spectrophotometrically A is found in a concentration of approximately 1 to 3 per cent in the blood of normal persons Levels in excess of 3 1 per cent have been shown to have diagnostic value for the thalassemia trait [131 136]

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years ago because of the sudden development of a hemolytic component after a burn necessitating periodic transfusions Since splenectomy the hemoglobin concentration is stabilized at 9 to 10 gm per 100 ml without transfusions although the blood smear still shows marked normoblastemia and other typical red cell changes characteristic of severe Cooleys anemia

The frequently made statement that thalassemia major is almost always fatal before puberty no longer holds within the accepted definition of this term It is difficult however to designate the precise period of puberty in these patients since the development of sexual maturity is greatly retarded Whereas infection was responsible for the death of many children with severe Cooley s anemia in the years before antibiotics were available they now succumb to heart failure Although long term transfusions prolong the lives of these patients they are nevertheless responsible in large part for increased tissue iron That excessive absorption of iron is also important as a cause of hemosiderosis is emphasized in those patients with thalassemia who have received minimal transfusions and eventually succumbed to heart disease [1] In large part cardiac complications are related to the heavy deposition of iron in the myocardium and are comparable in this respect to a similar incidence in idiopathic hemochromatosis [61] Once heart failure sets in it is irreversible and the maximal duration of life from the onset of symptoms is approximately one year The initial sign of a threatening cardiac nature in the asymptomatic patient is prolonged auricular ventricular conduction (P R interval) and the appearance of atrial premature contractions With manifest heart failure more serious atrial as well as ventricular arrythmias recurred

Acute benign nonspecific pericarditis has been described in thalassemia especially among patients who have been splenectomized [17] In all patients the pericarditis was self limited and ended in complete recovery The basis for this peculiar susceptibility is unknown

Treatment There is no specific treatment for patients with severe Cooley s anemia Iron copper liver extracts from the spleen pancreas and adrenal gland and other endocrine products steroids large dosages of vitamin B components plasma and cell extracts and x ray therapy have all been used without effect Since only questionable improvement has been reported with cobalt [11] it is not employed in routine treatment Cooleys anemia represents a hypochromic microcytic anemia in which iron therapy is without value as would be expected from the abundant iron stores already present

Transfusions Transfusions are mainly relied upon as treatment for patients with thalassemia and when they are excessive splenectomy is necessary Hemoglobin and red blood cell levels in children with severe Cooleys anemia continue to decline progressively so that supportive transfusions are required to keep the patients asymptomatic under the stress of normal activity Usually this necessitates the administration of blood at two- to three week intervals to maintain minimal levels Transfusions however required to support adequate hemoglobin levels carry the inherent drawback of increased iron deposition from degraded hemoglobin The hemoglobin level at which transfusions are indicated cannot be arbitrarily fixed but varies with the associated signs and symptoms in the individual patient As a guide to management patients with severe

A_2 is increased in patients with thalassemia minor with a range of 2 to 9 per cent and a mean of 5.11 ± 1.36 which exceeds the mean value in patients with thalassemia major and other miscellaneous diseases.[66] The elevation of the A_2 component serves therefore to distinguish patients with thalassemia minor from normal persons. Maximal values have occasionally been found in patients with intermediate forms of thalassemia and rarely in those with the severe disease. It should be emphasized that although the majority of patients with thalassemia minor show elevated A_2 concentrations a small percentage possess a normal concentration of this component. In this connection it has been pointed out[67] that the thalassemia gene that is not associated with an elevated A_2 fraction may also be responsible for the failure to interact with the gene for sickling hemoglobin C and hemoglobin E. Ordinarily the simultaneous presence of the thalassemia gene (presumably elevated A_2) in combination with the gene for sickling results in a severe anemia resembling homozygous rather than heterozygous sickle cell anemia. Two types of thalassemia have therefore been postulated based upon high and low A_2.

Although starch block electrophoresis provides definitive separation and measurement of A_2, hemoglobin paper electrophoresis by far the simpler method serves a similar function especially where this pigment is present in substantial amounts. In our laboratory the normal A_2 range with the paper method was 5 to 14.3 per cent as compared with 13.2 to 27 per cent in patients known to have heterozygous thalassemia.[68] Only these values for A_2 which lie above the limit of overlap in both paper and starch methods are diagnostic for the heterozygous thalassemia state.

Course and Prognosis. Patients with thalassemia minor live normally whether or not mild anemia is present. The outlook for patients with thalassemia major is poor but the course is not a uniform one and depends upon the grade of severity of the disease encountered in the individual patient. As has been stated previously thalassemia major falls into two classifications: the severe type in which the patients require transfusions to maintain hemoglobin levels commensurate with normal activity and a less severe one the so called intermediate type in which patients function effectively without transfusions maintaining hemoglobin levels of approximately 7 to 9 gm. per 100 ml. This separation can usually be made clinically but transitional cases occur in which hemoglobin concentrations of patients requiring frequent transfusions subsequently stabilize at adequate levels without further treatment. On the other hand patients with intermediate disease are observed who require no transfusions until there is exposure to infection or other noxious agents and more frequently for no apparent reason. From this point on transfusions may be required so frequently that splenectomy is considered necessary.

Multiple transfusions at carefully spaced intervals, splenectomy in selected patients and the liberal use of antibiotics permit a better prognosis for longevity in patients with thalassemia major than had heretofore been considered. Since the introduction of these combined measures is relatively recent their effect on ultimate longevity still has to be determined especially in patients with the intermediate type of disease. In either classification it is rare for severely affected patients to survive beyond the third decade. Among the patients with thalassemia major in our series the oldest is now 24 years old and among those with the intermediate type of disease there are three at least who are in their thirties. One of these now 37 years old originally reported on when he was an adolescent[10] has three children. This patient's spleen was removed three

whelming infection following splenectomy in patients with Cooley's anemia and other dyscrasias in childhood[66,91,105,1 5] In comparison with the necessary numbers of splenectomies the incidence is avowedly small Nevertheless close supervision for several years postoperatively is essential so that appropriate treatment may be instituted immediately in the event of an infection

There is no contraindication to tonsillectomy in patients with Cooley's anemia Minor coagulation defects may coexist with structural and functional abnormalities of the liver

Homozygous Hemoglobin C Disease

Homozygous hemoglobin C disease is a rare condition occurring principally in Negroes and occasionally in Caucasians

Essential Features Anemia is absent or mildly hemolytic the reticulocyte count is normal or slightly increased and bilirubin may be slightly elevated—features indicative of a compensated hemolytic process The red cells are normocytic or slightly microcytic and normochromic normoblasts appear occasionally and tetragonal crystals of hemoglobin have been observed in 2 per cent of the erythrocytes in one patient[43] Red cells show a decreased osmotic fragility Moderate erythrocytic hyperplasia is present in the bone marrow The striking feature of the blood film is the large number of target cells estimated at 40 to 90 per cent of all erythrocytes Paper electrophoresis reveals 100 per cent hemoglobin C which has the slowest mobility rate of all hemoglobins tested by this technique With rare exceptions fetal hemoglobin is not elevated above normal levels As in patients with other hereditary hemolytic syndromes the red cells of patients with homozygous hemoglobin C disease injected into a normal recipient show a shortened survival The exponential type of survival time curve indicates the random destruction of red cells regardless of their age[185] Splenomegaly is either moderate or very marked Abdominal pain arthralgia jaundice and cholelithiasis are uncommon The prognosis is good and transfusions are rarely necessary Splenectomy is occasionally carried out for relief of symptoms relating to pain over the splenic area[43]

Hemoglobin C Trait

The combination of hemoglobins A and C is found in the asymptomatic carrier who is identified by the reaction of his blood to electrophoresis and the *presence of numerous target cells in the blood smear In the person with the* trait the C pigment varies from 28 to 44 per cent sickling is absent fetal hemoglobin is normal and a mild hypochromia may be present The hemoglobin C trait is detected in about 2 per cent of the American Negro population

Hereditary Elliptocytosis and Hemoglobin C Trait Two children in a Negro family were found to have both elliptocytosis and hemoglobin C Each of these factors was traced to the respective parents In one child some of the target cells associated with the hemoglobin C trait were elliptical indicating the combined genetic effects in the same cells Anemia in one child was corrected by iron and the red cell survival was normal The combination of elliptocytosis and hemoglobin C trait does not show a summation effect[4]

Cooley's anemia do not require transfusions until hemoglobin levels drop between 6.5 and 7.5 gm per 100 ml at which point clinical symptoms frequently set in

In our experience maintaining hemoglobin levels below 6 gm per 100 ml is potentially hazardous from the standpoint of developing heart failure In patients with established cardiac difficulties the hemoglobin level should be maintained between 8 and 9 gm per 100 ml to prevent hypoxia In addition to transfusion hemosiderosis another deterrent to excessive blood administration is the possibility that multiple transfusions may depress endogenous hemoglobin synthesis and red cell function[1,8]

Until specific treatment becomes available the most serious problem encountered in the patient with severe Cooley's anemia is the prevention and control of congestive heart failure Treatment consists of the usual means of combating heart failure and controlling arrhythmias—namely digitalization diuretics low salt diet quinidine etc In addition small transfusions of packed red cells are given At present there is no effective measure to rid the heart of excessive iron Mobilization of iron stores achieved by repeated phlebotomies in patients with idiopathic hemochromatosis is precluded in patients with Cooley's anemia because of the constant need for maintaining adequate hemoglobin levels Chelating agents which increase urinary excretion of iron are important in the treatment of patients with conditions such as severe Cooley's anemia in whom massive iron loads exist[61]

Splenectomy In the severely affected person the need for splenectomy is clearly indicated when transfusion requirements are progressively increased and when the spleen becomes massive and burdensome causing discomfort and symptoms due to pressure Although efforts are made in the immediate presplenectomy period to vary the number and size of transfusions so as to defer splenectomy the blood requirements nevertheless become so extreme that decision for the operation is usually forced[1]

At this time there will usually be abundant evidence that a hemolytic factor has developed which shortens the life of normal transfused cells When there is doubt labeling donor cells with radioactive chromium will establish the rate at which transfused blood is being destroyed Although the increasing need for transfusions before splenectomy and the sharp reduction afterward confirms the elevation of an extracorpuscular hemolytic component it does not appear to alter the basic disease significantly[1,7] Retardation of growth and the incidence of congestive heart failure are not altered by removal of the spleen From available data splenectomy appears more effective in the older child but this may reflect a genetically milder disease permitting postponement until puberty Another explanation is that slowing of growth and diminished metabolic needs in adolescence permit the establishment of erythropoietic equilibrium at levels compatible with well being without supplementation by transfusions Despite the preference to delay splenectomy until adolescence it is frequently necessary to remove the spleen of the younger patient because of excessive transfusion needs

Recent experience has demonstrated a potential hazard of severe and over

Hemoglobin G

Hemoglobin G pigment moves more slowly than hemoglobin A and faster than hemoglobin S corresponding electrophoretically to the area in which fetal hemoglobin is found [4, 4] Its mobility coincides with fetal hemoglobin or migrates somewhat more slowly Hemoglobin G is not alkali resistant and can be differentiated from fetal hemoglobin in this manner [2, 3]

Hemoglobin G Trait In a family from the African Gold Coast in which the parents were homozygous for hemoglobins A and G respectively nine siblings were heterozygous (A G) as expected [4, 4] These persons with the trait presented no clinical or hematologic abnormalities

Homozygous Hemoglobin G Disease The father of the aforementioned family (GG) was asymptomatic and did not have anemia (hemoglobin 13 9 gm) and the blood smear was free of abnormalities Fetal hemoglobin was not present reticulocytes were 2 per cent and the serum bilirubin level was normal [4, 48]

Hemoglobin G-Sickle Cell Disease With heterozygous hemoglobin G sickle cell disease a moderately severe hemolytic anemia occurs with splenomegaly sickling increased osmotic resistance to hypotonic saline solution and a blood picture resembling sickle cell thalassemia disease [2, 3]

Hemoglobin G-Thalassemia An Italian patient with hemoglobin G-thalassemia possessed a blood picture and clinical findings somewhat more severe than those of patients with thalassemia minor The hemoglobin was 10 gm and the smear revealed hypochromic red cells target cells and basophilic stippling Other features included no elevation in fetal hemoglobin slight reticulocytosis a palpable spleen and moderately hyperplastic bone marrow The red cell survival was about two thirds of the normal [1] In an American family of Italian origin hemoglobin G hemoglobin S and thalassemia occurred together [1, 2] Hemoglobin G did not appear clinically important taken alone or in combination with hemoglobin S or thalassemia

Hemoglobin H

Three members of a Chinese family were found to have a hypochromic microcytic anemia with blood smears resembling thalassemia The electrophoretic pattern revealed an abnormal hemoglobin migrating more rapidly than normal hemoglobin (A) Other features included refractoriness to iron therapy reticulocytosis poikilocytosis intraerythrocytic inclusion bodies decreased osmotic fragility a shortened red cell survival and splenomegaly [9, 14] The abnormal hemoglobin accounted for 35 to 40 per cent of the total fetal hemoglobin was slightly increased and the remainder was normal hemoglobin It is of interest that this fast moving hemoglobin did not appear in either parent and that the father and daughter of one of the affected patients showed red cell changes consistent with thalassemia minor [14] In all subsequent reports the same failure to find hemoglobin H in either parent was observed

A similar blood picture interpreted as hemoglobin H-thalassemia disease has been described in two unrelated middle aged Filipino men Hemoglobin analysis in these cases also revealed a major component of normal hemoglobin and a minor component of hemoglobin H Fetal hemoglobin was normal [1-]

Hemoglobin D

Hemoglobin D is a rare hemoglobin which possesses an electrophoretic mobility identical with *sickle hemoglobin. It is distinguished by its much higher* solubility in reduced form than the latter in a comparable physical state. In contrast to homozygous hemoglobin S disease, gelling, tactoid formation, and sickling are absent. *Homozygous hemoglobin D disease has rarely been reported*.[1, 4] The anemia is slight, target cells are numerous, osmotic resistance to hypotonic saline solution is increased, and the erythrocyte life span is shortened.

The person *with the trait (hemoglobins A and D) is asymptomatic and reveals* no quantitative blood changes and no sickling. He can only be identified by the presence of hemoglobin D by electrophoresis. Hemoglobin D is not uncommon among *American Negroes, with a reported incidence of 0.4 per cent*.[1] Foci of hemoglobin D prevalence have been described in Algerian Moslems and Indians of North Central India.[4a]

Hemoglobin E Disease

Hemoglobin E disease was discovered during an investigation of a group of severely anemic patients from Thailand whose clinical and hematologic characteristics closely resembled those of patients with severe thalassemia. In some of these patients a mild course and survival into adult life suggested deviation from the uniformly severe case of thalassemia. A significant feature was the presence of the trait of thalassemia in only one parent instead of in both, which was to be expected in the usual case of the severe disease in a child. Investigation revealed that these patients possessed an additional hemoglobin designated as compound E, which was also present in the second parent. With paper electrophoresis it was possible to demonstrate that hemoglobin E had a mobility intermediate between hemoglobin C and hemoglobin S. Further studies [130] revealed the several categories of pure or homozygous hemoglobin E disease, hemoglobin E trait (hemoglobin A plus hemoglobin E), and thalassemia-hemoglobin E disease. A survey in connection with these studies revealed that hemoglobin E was found in 13 per cent of persons of Thai extraction but was not detected in any of the racially unmixed Chinese in that area.

Homozygous Hemoglobin E Disease. The clinical features include easy fatigability, mild arthralgia, and occasionally icterus. In the small group of cases in this category the spleen was slightly enlarged in only one patient and the liver in another. The hemoglobin level is moderately lowered, the erythrocyte levels are normal, reticulocytes are not increased, and target *cells are numerous* (25 to 60 per cent). Red cell constants indicate a microcytic hypochromic anemia. The red cells show an increased resistance to hemolysis in hypotonic saline solutions. The bone *marrow shows a mild erythrocytic hyperplasia*. Paper electrophoresis reveals 94 to 95 per cent hemoglobin E and up to 6 per cent fetal hemoglobin.

Hemoglobin E Trait. Persons with the trait are asymptomatic. Blood values are normal and target cells are not observed. Paper electrophoresis reveals two components, normal hemoglobin A and hemoglobin E. Normal amounts of fetal hemoglobin are demonstrated by alkali denaturation.

normal hemoglobin Sickling was absent and no hematologic or physical abnormalities were found [9, 189]

Two hemoglobin types have been observed in Liberian natives—one with a mobility corresponding to hemoglobin J and the other between hemoglobin J and A No cytologic abnormalities were found in either patient [146]

Hemoglobin M

The designation of hemoglobin M was proposed[16] for a spectroscopically abnormal methemoglobin first described by Horlein and Weber[86] in a family with hereditary methemoglobinemia They showed that the cyanosis was due to the presence of methemoglobin with an unusual spectral characteristic which was attributable to abnormality of the globin Subsequently Gerald[67] found that the hemoglobin from a patient with hemoglobin M disease could be resolved into two components identified electrophoretically and spectroscopically—one as hemoglobin A and the other as hemoglobin M The nature of the spectral changes indicates that some of the heme groups of the methemoglobin form react abnormally and others apparently normally

In contrast to the therapeutic effect of methylene blue and ascorbic acid in patients with the established forms of congenital and of drug induced types of methemoglobinemia in converting the pigment back to normal hemoglobin methemoglobinemia due to hemoglobin M is unaffected by this therapy Patients with the abnormal hemoglobin M have approximately 15 to 20 per cent of the total pigment in the form of methemoglobin and are seemingly unaffected by its presence [89]

Miscellaneous Abnormal Hemoglobins

Several additional hemoglobinopathies reported from isolated areas thus far have only local significance They are characterized by the following mobilities on the basis of paper electrophoresis at pH 8 6 Hemoglobin K moves faster than hemoglobin A and is so closely attached to the latter that it appears as its prolongation [1, 1] The abnormal component moves slower than hemoglobin J Hemoglobin L[1] migrates between hemoglobin S and hemoglobin G Hemoglobin N[18] moves faster than hemoglobin J but more slowly than hemoglobin H Hemoglobin O migrates between hemoglobin S and hemoglobin E Hemoglobin Q[19-a] migrates in largest part between hemoglobin A and hemoglobin S the remainder consisting of hemoglobin H In contrast to patients with other hemoglobins listed in the miscellaneous category the patient with hemoglobin Q disease suffered with a hypochromic anemia refractory to treatment

SICKLE CELL VARIANTS

Sickle Cell—Thalassemia Disease (Microdrepanocytic Anemia)

Sickle cell-thalassemia disease (microdrepanocytic anemia) accounts for most of the cases of sickle cell disease in white persons (usually of Greek or Italian origin) although it has been reported in American Negroes [1, 5] Such a patient usually inherits the gene for thalassemia from one parent and the gene for sickle cell hemoglobin from the other Rare exceptions[161] include instances

Increasing numbers of patients with hemoglobin H in combination with thalassemia minor (hemoglobin H-thalassemia) have been observed.[6, 11, 18, 94] Cases of hemoglobin H have been found not only in Chinese and Filipinos but also in Thai, Greek, Italian and Swedish patients. Although *devoid of hemoglobin H* parents and siblings in these reports present evidence of the thalassemia trait as well as of intraerythrocytic inclusion bodies resembling Heinz bodies. Further testing for increases of A hemoglobin is necessary to confirm the diagnosis of *thalassemia minor in these patients.*[4]

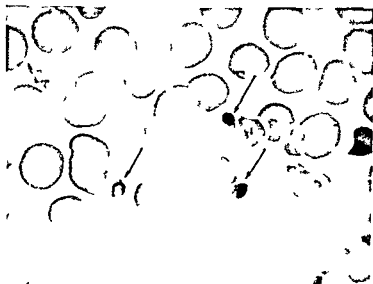


Fig. 25. Blood smear from a patient with hemoglobin H disease. Note inclusion bodies in three erythrocytes. (Courtesy Dr. Ralph L. Engle, Jr., New York, N. Y.)

Chronic hypochromic microcytic anemia associated with the presence of intra*erythrocytic crystals and hemoglobin H has been described. The parents did* not show the abnormal hemoglobin H in their blood. The family was partly of Chinese and partly of Indonesian origin.

Hemoglobin I

A hemoglobin with a mobility more rapid than normal hemoglobin A was detected in several members of a Negro family. The affected persons were asymptomatic and showed no hematologic abnormalities.[14] This hemoglobin was present in equal amounts with hemoglobin A. *Differences in mobility between hemoglobin* H and hemoglobin I were not apparent at a pH of 8.6 but were clearly distinguishable at a pH of 6.5.

Hemoglobin J

Hemoglobin J, observed in a Negro family, was shown to have a more rapid electrophoretic mobility at a pH of 8.6 than normal adult hemoglobin but a slower mobility than the next component I. It was found in combination with

is a moderate form of Cooley's anemia rarely of sickle cell anemia Anemia is moderate with hemoglobin levels of 6 to 9 gm per 100 ml usually about 8 gm and red cells number approximately 3 million per cubic millimeter Red cell constants indicate a microcytic hypochromic anemia as occurs in patients with thalassemia The other sickle cell variants show normochromic normocytic red cell constants as in homozygous (S S) sickle cell anemia

Clinical Findings The clinical manifestations are less severe than in patients with either sickle cell anemia or thalassemia major Crises are rare but abdominal bone and joint pain and at times unexplained fever occur Severe cases are occasionally observed as in patients with sickle cell anemia Jaundice is mild and splenomegaly is mild to moderate Aseptic necrosis of the head of the femur has also been reported[11] Splenomegaly is much more common than in patients with sickle cell anemia but not so marked as in those with severe Cooley's anemia Hepatomegaly may be of significant degree Patients with this disease are recognized because of anemia and later in childhood splenomegaly unlike those with thalassemia major in whom these features are already advanced in the first year of life Treatment depends upon severity In severe cases multiple transfusions are needed and splenectomy rarely if blood requirements are high Patients with mild cases require no treatment Prognosis is better than in patients with homozygous sickle cell anemia

Sickle Cell–Hemoglobin C Disease

The simultaneous presence of the sickle cell gene and the gene for hemoglobin C results in a type of sickle cell disease second in frequency only to classical sickle cell anemia among American Negroes[1, 4] The evidence derived from genetic studies strongly points to the association of these genes as alleles or close linkage upon the same chromosomes[149]

The clinical manifestations are usually of lesser severity than those in patients with homozygous sickle cell anemia but are extremely variable ranging from an asymptomatic state to severe disability Some of those patients heretofore regarded as having milder cases of sickle cell anemia probably belong in the category of hemoglobin S C disease Crises are of lesser severity and are infrequent hepatomegaly is usually present and splenomegaly is moderate or marked Fatigue dyspnea jaundice migratory arthralgia and recurrent abdominal pain may be present Pregnancy seems to aggravate the clinical and hematologic signs and carries a greater risk than classical sickle cell (S S) anemia Hematuria aseptic necrosis of the head of the femur and splenic infarction during aerial flights have been described in hemoglobin S C disease[180] The course varies markedly in different patients and in individual patients[19, b]

The anemia is usually of a mild normochromic type and the red cells vary slightly in size being either normal or decreased in volume The hemoglobin level rarely falls below 8 gm usually ranging from 9 to 10 gm and red cells range between 3 5 and 4 5 million per cubic millimeter Anisocytosis and poikilocytosis are occasionally noted in patients with severe disease Crises are associated with normoblastosis reticulocytosis and increased numbers of white cells in the peripheral blood The reticulocyte count generally is normal or slightly elevated

in which one parent with sickle cell-thalassemia disease (the other parent being normal) transmits both of the abnormal factors to the offspring In another instance a normal offspring resulted from one parent with the combined disease and the other normal Electrophoretic studies reveal the presence of sickle normal and occasionally fetal hemoglobin The percentage of sickle hemoglobin ranges from 65 to 80 per cent (in one patient 90 per cent) which is less than the 75 to more than 85 per cent present in homozygous sickle cell anemia[1] Hemoglobin analyses by electrophoresis and alkali denaturation have demonstrated that thalassemia-sickle cell disease consists of hemoglobins S A and F or hemoglobins S and A *Homozygous sickle cell anemia on the other hand* consists exclusively of sickle and fetal hemoglobins the latter in amounts of 2 to 24 per cent[161] The higher percentage of sickle cell hemoglobin in the offspring than in the parent with the trait (25 to 45 per cent hemoglobin S) reflects the potentiating effect of the associated thalassemia gene The disproportionate amount of hemoglobin S despite its presence in only one parent may also stem from the depression of normal hemoglobin synthesis by the thalassemia gene with corresponding increase of the abnormal hemoglobin The clinical manifestations of the combined disease represent predominantly a sickle type of hemolytic anemia although of lesser severity than the homozygous sickle cell disease

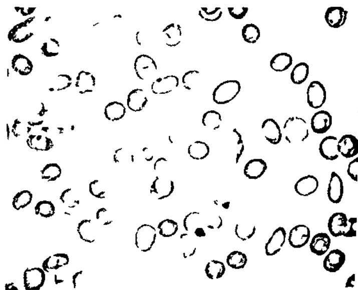


Fig 26 Blood smear from a patient with sickle cell thalassemia disease (×700) Note hypochromic macrocytes and tendency for oval cell target cells and sickle cell to occur

Essential Features The blood smear shows thin hypochromic microcytes poikilocytosis marked anisocytosis oval cells basophilic stippling and less frequently normoblasts The increase in target cells exceeds that in patients with sickle cell anemia Sickle cells are few in number in the fixed smear but are increased at times to 100 per cent in sickle cell preparations Osmotic fragility is decreased Clinically and hematologically the disease presents itself

hemoglobin has been found. The liver and spleen may not be palpable or both organs may be greatly enlarged. Sickle cell hemoglobin D disease has been reported in an 18 year old Negro male.[111]

THALASSEMIA VARIANTS

Thalassemia–Hemoglobin C Disease

Thalassemia-hemoglobin C disease usually occurs in Negroes and exceptionally in white persons. The anemia varies in intensity; it may be mild, rarely severe—in which case a hemoglobin level of 7 to 9.5 gm. is observed. In the latter instance it runs the course of a severe chronic hypochromic microcytic anemia although milder than that in patients with the homozygous thalassemia disease. Usually

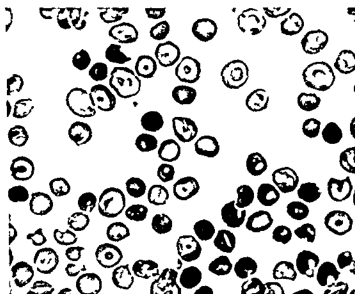


Fig. 27. Blood smear from patients with thalassemia-hemoglobin C disease. Note large number of target cells and microspherocytes. (From Erlandson, M., Smith, C. H., and Schulman, I.: Thalassemia-Hemoglobin C Disease in White Siblings, Pediatrics 17:740, 1956.)

however, there is no anemia and reticulocyte count is not elevated. Osmotic fragility may be normal but in a few cases increased resistance to hypotonic saline solution has been noted. The blood smear shows many target cells (20 to 60 per cent), anisocytosis and poikilocytosis, and an appreciable number of microspherocytes.[7] Since the percentage of target cells in patients with severe Cooley's anemia is usually less than 10 per cent, the finding of an unusually large percentage of these cells should lead one to suspect a hemoglobin variant of this disease. Reticulocytes number 2 to 5 per cent. Fetal hemoglobin is not elevated. Hepatosplenomegaly is either absent or moderate. The disease results from the interaction of the genes for sickling and thalassemia inherited from respective parents bearing the trait. In this combination also hemoglobin C usually is present in

serum bilirubin is normal and osmotic fragility is decreased. Erythroid hyperplasia which characterizes the bone marrow is not so marked as in the bone marrow of patients with homozygous sickle cell anemia.[101] The absence of anemia in many children reflects compensatory hyperactivity of the bone marrow and is indicated by an increased reticulocyte level. Sickle cells are few or absent in the blood smear but rapid and complete sickling occurs in sealed fresh preparations. Target cells are numerous, averaging 60 per cent (40 to 85 per cent). This feature is of some significance since excessive numbers of target cells in the blood smear of a patient with a mild form of sickle cell anemia should arouse suspicion of the coexistence of hemoglobin C.

In this disorder in which the patient is heterozygous for both abnormal hemoglobins, each of the components may be regarded as equally divided,[148] although hemoglobin C may range from 37 to 67 per cent. Fetal hemoglobin has been found in amounts up to 7 per cent. Transfusions may be required in episodes of severe hemolytic anemia and splenectomy has been employed in some of the patients in whom splenic infarction developed during flight.[181]

Sickle Cell—Hereditary Spherocytosis

Sicklemia in combination with congenital spherocytosis has been observed with improvement of the anemia following splenectomy.[37] Sickling remained unaffected with a residual blood pattern showing the sickle cell trait (hemoglobin A S). Each of the constitutional red cell defects (spherocytes and sickling) could be traced to individual patients.[98, 118, 180]

Hereditary Spherocytosis, Sickling and Thalassemia. An American Negro family has been described[8] in whom was found the genes responsible for three inherited abnormalities of the erythrocytes: spherocytosis, a thalassemia-like trait and the sickling phenomenon. One patient was the possessor of the three abnormal genes. Two (spherocytosis and sickling) were observed in blood examination, whereas the presence of the third (thalassemia) was inferred from its occurrence in two of the patient's children. The A₂ hemoglobin in this family was not increased. Analysis of the family pedigree suggests an independent and nonallelic segregation of the genes for each condition.

Sickle Cell–Hemoglobin D Disease

The patients with sickle cell hemoglobin D disease are white persons in whom the coexistence of hemoglobins S and D results in a moderately severe hemolytic process similar to that observed in patients with sickle cell anemia.[191] However, one parent reveals the sickle cell trait and the other the trait of hemoglobin D. The blood count reveals a moderate anemia with a hemoglobin level of about 8 to 9 gm. per 100 ml. of blood and a red cell count of approximately 2.5 million per cubic millimeter. The peripheral blood may show poikilocytosis, anisocytosis, polychromasia and occasionally nucleated red cells. In two reported patients[19] the red cells were increased in size. A few partially sickled cells are found in the fixed smear. With sickling tests the holly leaf or slow variety of sickling occurs rather than the filamentous or rapid variety found in patients with classical sickle cell anemia. Target cells are infrequent. Up to 12 per cent fetal

cytic hyperplasia Hemoglobin analysis shows a mixture of hemoglobins E and F with the former comprising 60 to 80 per cent of the total and fetal hemoglobin the remainder When complete studies have been available one parent possessed hemoglobin E and the other showed hematologic evidences of the thalassemia trait

Transfusions are necessary in many of the younger children but for the entire group they are not so urgent as in patients with severe Cooley s anemia Splenectomy in many of these patients has been of benefit in promoting growth and better general health

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Variants of thalassemia with hemoglobins G and H are described elsewhere in this book

Thalassemia—Lepore Hemoglobin

In the patient with the Lepore trait the blood picture resembles that of the patient with the trait of thalassemia (slight hypochromia microcytosis and leptocytosis) except for a low content of A hemoglobin The abnormal (Lepore) hemoglobin is detected by starch electrophoresis and not by paper electrophoresis At a pH of 8 6 it migrates in the region of sickle hemoglobin It represents 10 to 12 per cent of the total pigment In the cases reported[9] patients inherited the Lepore and the thalassemia traits from the respective parents The Lepore trait-thalassemia trait interaction results in a moderately severe hemolytic anemia with marrow hypertrophy secondary bone changes and moderate splenomegaly but not so extreme as in patients with thalassemia major The hemoglobin level ranges between 6 and 10 gm per 100 ml and the erythrocyte morphology resembles that of thalassemia consisting of marked microcytosis hypochromia and many target cells[1 6]

REFERENCES

1 Ager J A M and Lehmann H Haemoglobin L, a New Haemoglobin Found in a Punjabi Hindu Brit M J 2 142 1957

1a Ager J A M and Lehmann H Observations on Some Fast Haemoglobins K J N and Bart Brit M J 1 929 1958

1b Allison A C Protection Afforded by Sickle-Cell Trait Against Subtertian Malarial Infection Brit M J 1 290 1954

2 Allison A C Notation for Hemoglobin Types and Genes Controlling Their Synthesis Science 122 640 1955

2a Arend T Layrisse M and Rincon A R Sickle Cell-Haemoglobin D Disease in a Portuguese Child Acta haemat 22 118 1959

3 Astaldi G Ronanelli E G Bernardelli E and Strosselli E An Abnormal Substance Present in the Erythroblasts of Thalassemia Major Cytochemical Investigations Acta haemat 12 145 1954

4 Avery M E Hereditary Elliptocytosis and Hemoglobin C Trait a Report of Two Cases Bull Johns Hopkins Hosp 98 184 1956

5 Bailey I S and Prankerd T A J Studies in Thalassemia Brit J Haemat 4 150 1958

6 Banks, L O and Scott R B Thalassemia in Negroes Report of a Case of Cooley Anemia in a Negro Child Pediatrics 11 622 1953

7 Bannerman R M Grinstein M and Moore C V Haemoglobin Synthesis in Thalassemia in Vitro Studies Brit J Haemat 5 102 1959

an amount greatly in excess of that found in the parent and reflects the effect of coexistence with the thalassemia gene [163] In the reported patients [7, 163, 07] hemoglobin C ranged from 29 to 93 per cent

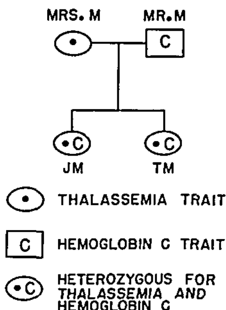


Fig 28 Example of a family pedigree showing genetic transmission of double heterozygosity for two different sets of genes—thalassemia and hemoglobin C (From Smith C H The Abnormal Hemoglobins Clinical and Hematologic Aspects J Pediat 50 91 1957 based on data from Erlandson M Smith C H and Schulman I Thalassemia–Hemoglobin C Disease in White Siblings Pediatrics 17 740 1956)

Thalassemia—Hemoglobin E Disease [7, 186]

Thalassemia-hemoglobin E disease represents a severe hemolytic syndrome simulating advanced Cooley s anemia (thalassemia major) so closely as to be indistinguishable from it except that the course may be somewhat milder Most cases of the combined disease have their onset in infancy although medical attention may not be sought until adolescence Fatigue dyspnea on effort joint pains pallor and jaundice occur frequently The abdomen is markedly protuberant the extremities are wasted and jaundice varies in severity Splenomegaly may be massive and hepatomegaly is less marked The patients are of small stature and present a facial appearance with mongoloid features similar to those of patients with classical Cooley s anemia

Hematologic examination reveals a microcytic hypochromic hemolytic anemia with markedly lowered hemoglobin and red cell values The reticulocyte count is elevated The blood smear coincides with that of the patients with the correspondingly severe Cooley s anemia In some patients the stained blood smear reveals target cells numbering 5 to 20 per cent of the red cells and a small number of spherocytes Although osmotic fragility starts in more concentrated solutions of sodium chloride than is normal fragility is not complete until the lowest dilutions are reached Nucleated red cells are prominent in the blood smear The bone marrow like that in patients with Cooley s anemia shows marked erythro

30 Cooley J C Peterson W L Engle C E and Jernigan J P Clinical Triad of Massive Splenic Infarction, Sicklemia Trait and High Altitude Flying J.A.M.A 154 111 1954

31 Cooley T B and Lee P A Series of Cases of Splenomegaly in Children With Anemia and Peculiar Bone Changes Tr Am Pediat Soc 37 29 1925

32 Cooley T B Witwer E R and Lee P Anemia in Children With Splenomegaly and Peculiar Changes in the Bone Report of Cases Am J Dis Child 34 347 1927

33 Cooper C D and Wacker W E C The Successful Therapy With Streptokinase Streptodornase of Ankle Ulcers Associated With Mediterranean Anemia Blood 9 241 1954

34 Crosby W H and Sacks H J The Coincidence of Mediterranean Anemia and Pernicious Anemia in a Young Sicilian Blood 4 1267 1949

35 Cummer C L and La Rocco C G Ulcers of the Legs in Sickle Cell Anemia Arch Dermat 42 1015 1940

36 Dacie J V The Haemolytic Anaemias Congenital and Acquired New York 1954 Grune & Stratton Inc

37 Dameshek W "Target Cell Anemia an Erythroblastic Type of Cooley s Erythroblastic Anemia Am J M Sc 200 445 1940

38 Dameshek W "Thalassemia" or What s in a Name Blood 10 293 1955

39 de Torregrosa M V Ortiz A., and Vargas D Sickle Cell Spherocytosis Associated With Hemolytic Anemia Blood 11 260 1956

40 Diggs L W Siderofibrosis of the Spleen in Sickle Cell Anemia J.A.M.A 104 538 1935

41 Diggs L. W The Crisis in Sickle Cell Anemia Hematologic Studies Am J Clin Path 26 1109 1956

42 Diggs L W and Ching R E Pathology of Sickle Cell Anemia South M J 27 839 1934

43 Diggs L W Kraus A P Morrison D B and Rudnicki R P T Intraerythrocytic Crystals in a White Patient With Hemoglobin C in the Absence of Other Types of Hemoglobin Blood 9 1172 1954

44 Diggs L W Pulliam H N and King J C The Bone Changes in Sickle Cell Anemia South M J 30 249 1937

45 Doenges J P Smith E W Wise S P and Breitenbucher R P Splenic Infarction Following Air Travel and Associated With the Sickling Phenomenon J.A.M.A 156 955 1954

46 Donegan C C Jr MacIlwaine W A and Leavell B S Hematologic Studies on Patients With Sickle Cell Anemia Following Multiple Transfusions Am J Med 17 29 1954

47 Edington G M and Lehmann A Haemoglobin G a New Haemoglobin Found in a West African Lancet 2 173 1954

48 Edington G M Lehmann H and Schneider R G Characterization and Genetics of Haemoglobin G Nature 175 850 1955

49 Editorial Sickle Cell Anemia in Africa Brit M J 2 433 1952

50 Editorial Sickling and Malaria Brit M J 2 310 1955

51 Ellis J T Schulman I and Smith C H Generalized Siderosis With Fibrosis of Liver and Pancreas in Cooley s (Mediterranean) Anemia Am J Path 30 287 1954

52 Eng L I Hin P S Kong K L and Endenburg P M Chronic Hypochromic Microcytic Anaemia Associated With Haemoglobin H Acta haemat 18 156 1957

52a Eng L I L and Sadono Haemoglobin O (Buginese X) in Sulawesi Brit M J 1:1461 1958

53 Erlandson M E and Hilgartner M Hemolytic Disease in the Neonatal Period and Early Infancy J Pediat 54 566 1959

54 Erlandson M E Schulman I and Smith C H Studies on Congenital Hemolytic Syndromes Rates of Destruction and Production of Erythrocytes in Sickle Cell Anemia Pediatrics 25 629 1960

8 Barrett A M Special Form of Erythrocyte Possessing Increased Resistance to Hypotonic Saline J Path & Bact 46 603 1938
9 Baty J M Blackfan K D and Diamond L K Blood Studies in Infants and in Children Erythroblastic Anemia Am J Dis Child 43 667 1932
9a Beavens G H Ellis M J and White J C Studies on Human Foetal Haemoglobin I Detection and Estimation Brit J Haemat 6 1 1960
10 Beet E A Sickle Cell Disease in the Balovale District of Northern Rhodesia E African M J 23 75 1946
11 Berk L Burchenal J H and Castle W B Erythropoietic Effect of Cobalt in Patients With and Without Anemia New England J Med 240 754 1949
12 Bianco I Moutalenti G Silvestroni E and Siniscalco M Further Data on Genetics of Microcythaemia or Thalassemia Minor and Cooley's Disease or Thalassemia Major Ann Eugenics 16 299 1952
12a Bird G W C and Lehmann H Haemoglobin D in India Brit M J 1 4 1956
13 Bridgers W H Cerebral Vascular Disease Accompanying Sickle Cell Anemia Am J Path 15 353 1939
14 Brinkman R and Jonxis J H P The Occurrence of Several Kinds of Haemoglobin in Human Blood J Physiol 85 117 1935
15 Bunting H Sedimentation Rates of Sickled and Non sickled Cells From Patients With Sickle Cell Anemia Am J M Sc 198 191 1939
15a Cabannes R and Buhr J L L'hemoglobine K identification incidence biologiques et pathologiques Sang 29 201 1958
16 Caffey J Cooley's Erythroblastic Anemia Some Skeletal Findings in Adolescents and Young Adults Am J Roentgenol 65 547 1951
17 Caffey J Pediatric X ray Diagnosis Chicago 1956 Year Book Publishers Inc
18 Caffey J Cooley's Anemia a Review of the Roentgenographic Findings in the Skeleton Am J Roentgenol 78 381 1957
19 Callender S T E Nickle J F Moore C V and Powell E O Sickle Cell Disease Studied by Measuring the Survival of Transfused Red Blood Cells J Lab & Clin Med 34 90 1949
20 Carrington H T Ferguson A D and Scott R B Studies in Sickle Cell Anemia Bone Involvement Simulating Aseptic Necrosis A M A J Dis Child 95 157 1958
21 Chaplin H Jr Keitel H G and Peterson R E Hematologic Observations on Patients With Sickle Cell Anemia Sustained at Normal Hemoglobin Levels by Multiple Transfusions Blood 11 834 1956
22 Chapman A Z Reeder P S Freidman I A and Baker L A Gross Hematuria in Sickle Cell Trait and Sickle Cell Hemoglobin C Disease Am J Med 19 773 1955
23 Chernoff A I Studies on Abnormal Hemoglobins Persistence of Fetal Hemoglobin in the Erythrocytes of Normal Children Pediatrics 9 469 1952
24 Chernoff A I The Human Hemoglobins in Health and Disease New England J Med 253 322 365 415 1955
24a Chernoff A I The Hemoglobin D Syndromes Blood 13 116 1958
25 Chernoff A I Shapleigh J B and Moore C V Therapy of Chronic Ulceration of the Legs Associated With Sickle Cell Anemia J A M A 155 1487 1954
26 Chernoff A I The Distribution of the Thalassemia Gene a Historical Review Blood 14 899 1959
27 Chernoff A I Minnich V Na Nakorn S Tuchinda S Kashemsant C and Chernoff R R Studies on Hemoglobin E the Clinical and Genetic Characteristics of the Hemoglobin E Syndromes J Lab & Clin Med 47 455 1956
28 Cohen F Zuelzer W W Neel J V and Robinson A R Multiple Inherited Erythrocyte Abnormalities in an American Negro Family Hereditary Spherocytosis Sickling and Thalassemia Blood 14 816 1959
29 Conn H O Sickle Cell Trait and Splenic Infarction Associated With High Altitude Flying New England J Med 251 417 1954

79 Hedenberg F Muller Eberhard U Sjolin S and Wranne L Haemoglobin H and Inclusion Body Anaemia in a Swedish Family Acta paediat 47 652 1958
80 Henkin W A Collapse of the Vertebral Bodies in Sickle Cell Anemia Am J Roentgenol 62 395 1949
81 Henry M D and Chapman A Z Vitreous Hemorrhage and Retinopathy Associated With Sickle Cell Disease Am J Ophth 38 204 1954
82 Herrick J B Peculiar Elongated and Sickle Shaped Red Blood Corpuscles in a Case of Severe Anemia Arch Int Med 6 517 1910
83 Hilgartner M W Erlandson M E Walden B S and Smith C H A Comparison of the Paper and Starch Block Electrophoretic Methods for A Hemoglobin *Program of* Society for Pediatric Research twenty ninth Annual Meeting 1959 p 36
84 Hill F S Hughes J G and Davis B C *Electro encephalographic Findings in Sickle* Cell Anemia Pediatrics 6 277 1950
85 Hook E W Campbell C G Weens H S and Cooper G R Salmonella Osteomyelitis in Patients With Sickle Cell Anemia New England J Med 257 403 1957
86 Horlein H and Weber G Ueber chronische Familiare Methamoglobinamie und eine neue Modifikation des Methamoglobins Deutsche med Wchnschr 73 476 1948
87 Howell J and Wyatt J P Development of Pigmentary Cirrhosis in Cooley s Anemia A M A Arch Path 55 423 1953
88 Hsia D Y Y Inborn Errors of Metabolism Chicago 1959 Year Book Publishers Inc
89 Hughes J G and Carroll D S Salmonella Osteomyelitis Complicating Sickle Cell Disease Pediatrics 19 184 1957
90 Hughes J G Diggs L W and Gillespie C E The Involvement of the Nervous System in Sickle Cell Anemia J Pediat 17 166 1940
91 Huntley C C *Infection* Following Splenectomy *in Infants* and Children A M A J Dis Child 95 477 1958
92 Ingram V M Gene Mutations in Human Haemoglobin the Chemical Difference Between Normal and Sickle Cell Haemoglobin Nature 180 326 1957
93 Ingram V M Chemistry of the Abnormal Human Haemoglobins Brit M Bull 15 27 1959
94 Itano H A Bergren W R and Sturgeon P The Abnormal Human Haemoglobins Medicine 35 121 1956
95 Ivy R E and Howard F H Sickle Cell Anemia With Unusual Bone Changes J Pediat 43 312 1953
96 Jandl J H and Greenberg M S Bone Marrow Failure Due to Relative Nutritional Deficiency in Cooley's Hemolytic Anemia Painful Erythropoietic Crises in Response to Folic Acid New England J Med 260 461 1959
96a Jenkins M E Scott R B and Baird R L Studies in Sickle Cell Anemia XVI Sudden Death During Sickle Cell Anemia Crises in Young Children J Pediat 56 30 1960
97 Jenkins M E Scott R B and Ferguson A D Studies in Sickle Cell Anemia Blood Volume Relationships and the Use of a Plasma Extender in Sickle Cell Disease in Childhood a Preliminary Report Pediatrics 18 239 1956
97a Jim R T S and Yarbro M T Hemoglobin J in a Healthy Hawaiian Chinese Caucasian Male Blood 15 285 1960
98 Jim B and Mingle g W G Hemoglobin S Hereditary Spherocytosis J Pediat 54 375 1959
99 Jonsson U Roath O S and Kirkpatrick C I F Nutritional Megaloblastic Anemia Associated With Sickle Cell States Blood 14 535 1959
99a Jonxis J H P and Delafresnaye J F (editor) Abnormal Haemoglobins Springfield Ill 1959 Charles C Thomas Publisher
100 Jonxis J H P and Huisman T H J The Detection and Estimation of Fetal Hemoglobin by Means of the Alkali Denaturation Test Blood 11 1009 1956
101 Josephson A M Masri M S Singer L Dworkin D and Singer K Starch Block Electrophoretic Studies of Human Hemoglobin Solutions Results in Cord Blood Thalas

55 Erlandson M E Schulman I Stern G and Smith C H Studies of Congenital Hemolytic Syndromes Rates of Destruction and Production of *Erythrocytes in Thalassemia* Pediatrics 22 910 1958
56 Erlandson M E Schulman I Walden B and Smith C H Chromium[51] Elution From Hemoglobin and Intact Erythrocytes of Adults Infants and Patients With Cooley's *Anemia Proc Soc Exper Biol & Med 99:173 1958*
57 Erlandson M Smith C H and Schulman I Thalassemia Hemoglobin C Disease in White Siblings Pediatrics 17 740 1956
58 Estes J E Farber E M and Stickney J M Ulcers of the Leg in Mediterranean *Disease Blood 3 302 1948*
59 Etteldorf J N Smith J D Tuttle A H and Diggs L W Renal Hemodynamic Studies in Adults With Sickle Cell Anemia Am J Med 18 243 1955
60 Ferguson A D Carrington H T and Scott R B Studies in Sickle Cell Anemia a Clinical Review M Ann District of Columbia 24 517 1955
61 Finch S C and Finch C A Idiopathic Hemochromatosis an Iron Storage Disease Iron Metabolism in Hemochromatosis Medicine 34 381 1955
62 Foerster R F The Italian Emigration of Our Times Cambridge Mass 1919 Harvard University Press chap 17
63 Gerald P S The Electrophoretic and Spectroscopic Characterization of Hgb M Blood 13:936 1958
64 *Gerald P S and Diamond L K The Diagnosis of Thalassemia Trait by Starch Block* Electrophoresis of the Hemoglobin Blood 13 61 1958
65 Gerald P S and Diamond L K A New Hereditary Hemoglobinopathy (Lepore Trait) and Its Interaction With Thalassemia Trait Blood 13 835 1958
66 *Gofstein R and Gellis S S Splenectomy in Infancy and Childhood A M A J Dis* Child 91 566 1956
67 Goldberg M A and Schwartz S O Mediterranean Anemia in a Negro Complicated by Pernicious Anemia of Pregnancy Blood 9 648 1954
68 Gouttas A Fessas P H Tsevrenis H and Xefteri E Description d'une nouvelle variété d'anémie hémolytique congénitale Sang 26 911 1955
69 Green T W Conley C L and Berthrong M The Liver in Sickle Cell Anemia Bull Johns Hopkins Hosp 92 99 1953
70 Greenberg M S and Kass E H Studies on the Destruction of Red Blood Cells Observations on the Role of pH in the Pathogenesis and Treatment of Painful Crisis in Sickle Cell Disease A M A Arch Int Med 101 355 1958
71 Griggs R C and Harris J W The Biophysics of the Variants of Sickle Cell Disease A M A Arch Int Med 97 315 1956
72 Gross R T Kriss J P and Spaet T H The Hematopoietic Goitrogenic Effects of Cobaltous Chloride in Patients With Sickle Cell Anemia Pediatrics 15:284 1955
73 Hahn E V and Gillespie E B Sickle Cell Anemia Report of a Case Greatly Im*proved by Splenectomy Arch Int Med 39 233 1927*
74 Halbrecht I Studies on the Different Types of Hemoglobin in the Blood of the Newborn and Fetus With Special Reference to the New Embryonic (Primitive) Hemoglobin Sixth International Congress of the International Society of Hematology Boston 1956 p 433
75 Harden A S Sickle Cell Anemia Changes in the Vessels and in the Bones Am J Dis Child 54 1045 1937
76 *Harris J W Studies on the Destruction of Red Blood Cell* Molecular Orientation in Sickle Cell Hemoglobin Solutions Proc Soc Exper Biol & Med 75 197 1950
77 Harris J W The Role of Physical and Chemical Factors in the Sickling Phenomenon In Tocantins L M (editor) Progress in Hematology vol 2 New York 1959 Grune *& Stratton Inc p 47*
78 Harris J W Brewster H H Ham T H and Castle W B Studies on the Destruction of Red Blood Cells the Biophysics and Biology of Sickle Cell Disease A M A Arch Int Med 97 145 1956

125 Motulsky A G Genetic Haematological Significance of Haemoglobin H Nature 178 1055 1956
126 Motulsky A G Hebenstreit W Lonn L Erikson N and Laeth C W Hemoglobin H Thalassemia Disease and Further Studies on the Interaction of Thalassemia With Hb C and S Proceedings of Sixth Congress of International Society of Hematology New York 1958 Grune & Stratton Inc p 729
127 Motulsky A G Paul, M H and Durrum E L Paper Electrophoresis of Abnormal Hemoglobins and Its Clinical Applications Blood 9 897 1954
128 Muro P D and Leonardi G Hemopathic Mediterranean Syndrome Acta med scandinav 138 362 1950
129 Myerson R M Harrison E and Lohmuller H W Incidence and Significance of Abnormal Hemoglobins Report of a Series of 1 000 Hospitalized Negro Veterans Am J Med 26 543 1959
130 Na Nakorn S Minnich V and Chernoff A I Studies on Hemoglobin E the Incidence of Hemoglobin E in Thailand, J Lab & Clin Med 47 490 1956
131 Neel J V The Inheritance of Sickle Cell Anemia Science 110 64 1949
132 Neel J V and Valentine W N The Frequency of Thalassemia Am J M Sc 209 568 1945
133 Ohner H L and Heller P Megaloblastic Erythropoiesis and Acquired Hemolysis in Sickle Cell Anemia New England J Med 261 19 1959
134 Olshin I and Sawitsky A Familial Thalassemia and Erythremic Myelosis Pediatrics 22 250 1958
134a Pascher F and Keen R Ulcers of the Leg in Cooleys Anemia New England J Med 256 1220 1957
135 Pauling L Itano A H Singer S J and Wells J C Sickle Cell Anemia a Molecular Disease Science 110 543 1949
136 Pearson H A Gerald P S and Diamond L K Thalassemia Intermedia Due to Interaction of Lepore Trait With Thalassemia Trait Report of Three Cases A M A J Dis Child 97 464 1959
137 Perosa L Ramunni M Bini L and Manganelli G The Genesis of Sickle Cell Morphology Acta haemat 18 255 1957
138 Perutz M F and Mitchison J M State of Haemoglobin in Sickle Cell Anaemia Nature 166 677 1950
139 Plachta A and Spier R D The Coexistance of Rheumatic Heart Disease and Sickle Cell Anemia Review of the Literature and Report of Case Am J Clin Path. 22 970 1952
140 Ranney H M Observations on the Inheritance of Sickle Cell Hemoglobin and Hemoglobin C J Clin Invest 33 1634 1954
141 Raper A B Sickling in Relation to Morbidity From Malaria and Other Diseases Brit M J 1 965 1956
142 Reich R S and Rosenberg N J Aseptic Necrosis of Bone in Caucasians With Chronic Hemolytic Anemia Due to Combined Sickling and Thalassemia Traits J Bone & Joint Surg 35 A 894 1953
143 Reinhard E H Moore C V Dubach R and Wade L J Depressant Effects of High Concentrations of Inspired Oxygen on Erythrocytogenesis J Clin Invest 23 682 1944
144 Rich A Studies on the Hemoglobin of Cooleys Anemia and Cooleys Trait Proc Nat Acad Sc 38 187 1952
145 Rigas D A Koler R D and Osgood E E Hemoglobin H Clinical Laboratory and Genetic Studies of a Family With a Previously Undescribed Hemoglobin J Lab & Clin Med 47 51 1956
146 Robinson A R Zuelzer W W Neel J V Livingstone F B and Miller M J Two "Fast" Hemoglobin Components in Liberian Blood Samples Blood 11 902 1956
147 Rucknagel D L Page E B and Jensen W N Hemoglobin I an Inherited Hemoglobin Anomaly Blood 10 999 1955

semia and Other Hemolytic Disorders Comparison With Tiselius Electrophoresis Blood 13 543 1958
102 Kaplan E and Zuelzer W W Erythrocyte Survival Studies in Childhood Studies in Mediterranean Anemia J Lab & Clin Med 36 517 1950
103 Kaplan E Zuelzer W W and Neel J V Further Studies on Hemoglobin C the Hematologic Effects of Hemoglobin C Alone and in Combination With Sickle Cell Hemoglobin Blood 8 735 1953
104 Keitel H G Thompson D and Itano H A Hyposthenuria in Sickle Cell Anemia a Reversible Renal Defect J Clin Invest 35 998 1956
105 King H and Shumacker H B Jr Splenic Studies Susceptibility to Infection After Splenectomy Performed in Infancy Am J Surg 136 239 1952
106 Klinefelter H F The Heart in Sickle Cell Anemia Am J M Sc 203 34 1942
107 Korber E Cited by Bischoff H Z Resistance of Human Hemoglobin With Especial Regard to Infants Ztschr ges exper Med 48 472 1926
108 Kunkel H G Ceppelini R Muller Eberhard U and Wolf J *Observations on the* Minor Basic Hemoglobin Component in the Blood of Normal Individuals and Patients With Thalassemia J Clin Invest 36 1615 1957
109 Kunkel H G and Wallenius G New Hemoglobin in Normal Adult Blood Science 122 288 1955
110 Kunz H W Pratt E I Mellin G W and Cheung M W Impairment of Urinary Concentration in Sickle Cell Anemia Pediatrics 13 352 1954
111 Leavell B S Treatment of Sickle Cell Anemia A M A Arch Int Med 94 801 1954
112 Lehman H and Cutbush M Sickle Cell Trait in Southern India Brit M J 1 404 1952
113 Lerner A B Melanin Pigmentation Am J Med 19 902 1955
114 Levin W C Asymptomatic Sickle Cell Trait (editorial) Blood 13 904 1958
115 Liachowitz C Elderkin J Guichirit I Brown H W and Ranney H M Abnormal Hemoglobins in the Negroes of Surinam Am J Med 24 19 1958
116 Lichtman H C Shapiro H Ginsberg V and Watson R J Splenic Hyperfunction in Sickle Cell Anemia (Abstr) Am J Med 14 516 1953
116a Lieb W A Geeraets W J and Guerry D III Sickle Cell Retinopathy Ocular and Systemic Manifestations of Sickle Cell Disease Acta ophth suppl 58 7 1959
117 Liquori A M Presence of Fetal Hemoglobin in Cooley s Anemia Nature 167 950 1951
118 Margolies M P Sickle Cell Anemia Composite Study and Survey Medicine 30 357 1951
118a Martin W W Jr Kough R H and Branche G C Jr Hereditary Spherocytosis—Sicklemia in the Negro Case Report and Study of a Negro Family Having Multiple Instances of Hereditary Spherocytosis Blood 14 688 1959
119 Matoth Y Shamir Z and Freundlich E Thalassemia in Jews From Kurdistan Blood 10 176 1955
119a McCurdy P R Clinical and Physiologic Studies in a Negro With Sickle Cell Hemoglobin D Disease New England J Med 262 961 1960
120 McElfresh A E Sharpsteen J R and Akabane T Secondary Hypersplenism Occurring in a Seven Month Old Infant with Thalassemia Major J Pediat 47 347 1955
121 Minnich V Na Nakorn S Chongchareonsuk S and Kochaseni S Mediterranean Anemia a Study of Thirty Two Cases in Thailand Blood 9 1 1954
122 Minnich V Na Nakorn S Tuchinda S Iravit W and Moore C V Inclusion Body Anemia in Thailand (Hemoglobin H Thalassemia Disease) Proceedings of Sixth Congress of International Society of Hematology New York 1958 Grune & Stratton Inc p 743
122a Moseley J I Pattern of Bone Change in Sickle Cell States J Mount Sinai Hosp 26 424 1959
123 Moser K M and Shea J G The Relationship Between Pulmonary Infarction Cor Pulmonale and the Sickle State Am J Med 22 561 1957
124 Motulsky A G Sicklemia J A M A 155 388 1954

171 Smith C H Familial Blood Studies in Cases of Mediterranean (Cooley s) Anemia a Diagnosis of the Trait or Mild Form of the Disease Am J Dis Child 65 681 1943
172 Smith C H Detection of Mild Types of Mediterranean (Cooley s) Anemia Am J Dis Child 75 505 1948
173 Smith C H Transfusions in Pediatrics Practice Indications and Limitations Pediatrics 17 596 1956
174 Smith C H Erlandson M E Schulman I and Stern G Serum Iron and Iron Binding Capacity of the Serum in Children With Severe Mediterranean (Cooley s) Anemia Pediatrics 5 799 1950
175 Smith C H Erlandson M E Schulman I and Stern G Hazard of Severe Infections in Splenectomized Infants and Children Am J Med 22 390 1957
175a Smith C H Erlandson M E Stern G and Schulman I The Role of Splenectomy in the Management of Thalassemia Blood 15 197 1960
176 Smith C H and Morgenthau J E Cholelithiasis in Severe Mediterranean (Cooley s) Anemia Blood 6 1147 1951
177 Smith C H Schulman I Ando R E and Stern G Studies in Mediterranean (Cooley s Anemia Clinical and Hematologic Aspects of Splenectomy With Special Reference to Fetal Hemoglobin Synthesis Blood 10 582 1955
178 Smith C H Schulman I Ando R E and Stern G Studies in Mediterranean (Cooley s) Anemia the Suppression of Hematopoiesis by Transfusions Blood 10 707 1955
179 Smith E Rosenblatt P and Bedo A V Sickle Cell Anemia Crisis Report on Seven Patients Treated With Priscoline J Pediat 43 655 1953
180 Smith E W and Conley C L Clinical Features of the Genetic Variants of Sickle Cell Disease Bull Johns Hopkins Hosp 94 289 1954
181 Smith E W and Conley C L Sicklemia and Infarction of the Spleen During Aerial Flight Bull Johns Hopkins Hosp 96 35 1955
181a Smith E W and Conley C L Sickle Cell Hemoglobin D Disease Ann Int Med 50 94 1959
182 Spaet T H Personal communication New York
183 Sprague C C and Paterson J C S Role of the Spleen and Effect of Splenectomy in Sickle Cell Disease Blood 13 569 1958
184 Sturgeon P and Finch C A Erythrokinetics in Cooley s Anemia Blood 12 64 1957
185 Sturgeon P Itano H A and Bergren W R Clinical Manifestations of Inherited Abnormal Hemoglobins the Interactions of Hemoglobin S With Hemoglobin D Blood 10 389 1955
186 Sturgeon P Itano H A and Bergren W R Clinical Manifestations of Inherited Abnormal Hemoglobin Interaction of Hemoglobin E and Thalassemia Trait Blood 10 396 1955
187 Tanaka K R Clifford G O and Axelrod A R Sickle Cell Anemia (Homozygous S) With Aseptic Necrosis of the Femoral Head Blood 11 998 1956
188 Thomas E D Motulsky A G and Walters D H Homozygous Hemoglobin C Disease Report of a Case With Studies on the Pathophysiology and Neonatal Formation of Hemoglobin C Am J Med 18 832 1955
189 Thorup O A Itano H A Wheby M and Leavell B S Hemoglobin J Science 123 889 1956
190 Todd R McC and O Donohue N V Acute Acquired Haemolytic Anaemia Associated With Herpes Simplex Infection Arch Dis Childhood 33 524 1958
191 Thomlinson W J Abdominal Crisis in Uncomplicated Sickle Cell Anemia a Clinico-Pathologic Study of 11 Cases With a Suggested Explanation of Their Cause Am J Med 209 722 1945
192 Trowell H C Raper A B and Welbourn H F The Natural History of Homozygous Sickle Cell Anaemia in Central Africa Quart J Med 26 401 1957
192a Vella F Wells R H C Ager J A M and Lehmann H A Haemoglobinopathy Involving Haemoglobin H and a New (Q) Haemoglobin Brit M J 1 752 1958

148 Schell, N. B., and McCurdy, J. M.: Sickle-Cell Hemoglobin C Disease. A.M.A. J. Dis. Child. 91:38, 1956.
149 Schulman, I., Smith, C. H., and Stern, G. S.: Studies on the Anemia of Prematurity. A.M.A. Am. J. Dis. Child. 88:567, 1954.
150 Schwartz, H., and Spaet, T. H.: Hemoglobin G, a Fifth Abnormal Hemoglobin. Clin. Res. Proc. 3:51, 1955.
150a Schwartz, H. C., Spaet, T. H., *Zuelzer, W. W., Neel, J. V., Robinson, A. R.*, and Kaufman, S. F.: Combination of Hemoglobin G, Hemoglobin S and Thalassemia Occurring in One Family. Blood 12:238, 1957.
151 Scott, R. B., in Cooke, S. S. *(editor): Year Book of Pediatrics, Series 1957-1958.* Chicago, 1957, Year Book Publishers, p. 2[illegible].
152 Scott, R. B., Banks, L. O., Jenkins, M. E., and Crawford, R. P.: Studies in Sickle Cell Anemia: Clinical Manifestations of Sickle-Cell Anemia in Children. J. Pediat. 39:460, 1951.
153 Scott, R. B., Ferguson, A. D., Jenkins, M. E., and Clark, H. M.: Studies in Sickle Cell Anemia: Further Observations on the Clinical Manifestations of Sickle Cell Anemia in Children. A.M.A. Am. J. Dis. Child. 90:682, 1955.
154 Scott, R. B., and Jenkins, M. E.: Studies in Sickle Cell Anemia: Sickle-Cell Hemoglobin C Disease. A.M.A. Am. J. Dis. Child. 90:35, 1955.
155 Shelley, W. M., and Curtis, E. M.: Bone Marrow and Fat Embolism in Sickle Cell Anemia and Sickle Cell-Hemoglobin C Disease. Bull. Johns Hopkins Hosp. 103:8, 1958.
156 Shen, S. C., Fleming, E. M., and Castle, W. B.: Studies on the Destruction of Red Blood Cells: Irreversibly Sickled Erythrocytes: Their Experimental Production in Vitro. Blood 4:498, 1949.
157 Shields, G. S., Lichtman, H. C., Messite, J., and Watson, R. J.: Studies in Sickle Cell Disease: Quantitative Aspects of Sickling in the Newborn Period. Pediatrics 22:309, 1958.
158 Shields, G. S., Wethers, D., Cave, G., and Watson, R. J.: Hemoglobin S-Thalassemia Disease. A.M.A. J. Dis. Child. 91:485, 1956.
159 Shotton, D., Crockett, C. L., Jr., and Leavell, B. S.: Splenectomy in Sickle Cell Anemia: Report of a Case and Review of the Literature. Blood 6:365, 1951.
160 Silver, H. K.: Mediterranean Anemia in Children of Non-Mediterranean Ancestry. Am. J. Dis. Child. 80:767, 1950.
161 Silvestroni, E., and Bianco, I.: Genetic Aspects of Sickle-Cell Anemia and Microdrepanocytic Disease. Blood 7:429, 1952.
162 Singer, K.: Hereditary Hemolytic Disorders Associated With Alterations of the Hemoglobin Molecule. Am. J. Med. 18:633, 1955.
163 Singer, K., Chapman, A. Z., Goldberg, S. R., Rubenstein, H. M., and Rosenblum, S. A.: Studies on Abnormal Hemoglobins: A New Syndrome: Hemoglobin C-Thalassemia Disease. Blood 9:1023, 1954.
164 Singer, K., Chernoff, A. I., and Singer, L.: Studies on Abnormal Hemoglobins. Blood 6:413, 1951.
165 Singer, K., and Fisher, B.: Studies on Abnormal Hemoglobins: Electrophoretic Demonstration of Type S (Sickle Cell) Hemoglobin in Erythrocytes Incapable of Showing the Sickle Cell Phenomenon. Blood 8:270, 1953.
166 Singer, K., Josephson, A. M., Singer, L., Heller, P., and Zimmerman, H. S.: Studies of Abnormal Hemoglobins: Hemoglobin S-Thalassemia Disease and Hemoglobin C-Thalassemia Disease in Siblings. Blood 12:593, 1957.
167 Singer, K., Robin, S., King, J. C., and Jefferson, R. N.: The Life Span of the Sickle Cell and the Pathogenesis of Sickle-Cell Anemia. J. Lab. & Clin. Med. 33:975, 1948.
168 Singer, K., and Singer, L.: Studies on Abnormal Hemoglobins: The Gelling Phenomenon of Sickle Cell Hemoglobin: Its Biologic and Diagnostic Significance. Blood 8:1008, 1953.
169 Singer, K., Singer, L., and Goldberg, S. R.: Studies on Abnormal Hemoglobins: Sickle Cell-Thalassemia Disease in the Negro. Blood 10:405, 1955.
170 Smith, C. H.: Mediterranean (Cooley's) Anemia in a Youth of Nineteen Years Observed Since Early Childhood. J. Pediat. 20:370, 1942.

CHAPTER 17

Polycythemia, Methemoglobinemia, Sulfhemoglobinemia, and Miscellaneous Anemias

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POLYCYTHEMIA

Polycythemia refers to an increase in the number of red blood cells in the hemoglobin level and in the hematocrit per unit volume of blood which is substantially in excess of normal values. Several types of polycythemia may be differentiated: *true or absolute polycythemia*, in which the total number of red cells (red cell mass) in the circulating blood is increased, and *relative polycythemia*, resulting from the shrinkage of the total plasma volume, in which the red cell volume remains normal.

True polycythemia may be further classified into *primary polycythemia* (polycythemia vera) of unknown etiology, and *secondary polycythemia*, in which there is an increase in total volume of red cells in response to a recognizable cause. The latter includes conditions in which oxygen saturation of arterial blood is lowered, pathologic states involving the central nervous system, and abnormalities of pigment metabolism. Except in persons with polycythemia due to hemoconcentration, the blood volume is increased chiefly as a result of augmentation of circulating red cells.

The alkaline phosphatase activity in the polymorphonuclear cell serves as a differential test between the two types of polycythemia. A strongly positive test is observed in persons with leukemoid reactions due to polycythemia vera, and a negative test signifies myelogenous leukemia. Secondary polycythemia unassociated with a leukemoid response falls within the range of normal.[6, 36]

Relative Polycythemia

Relative polycythemia is observed in persons with conditions of hemoconcentration associated with marked loss of body fluids, as occurs in patients with diarrhea, vomiting, severe burns, profuse sweating, and dehydration from water deprivation. The "polycythemia of stress," occurring in anxiety states in adults, is also due to reduced blood volume.[3, 7] In patients with relative polycythemia the white blood cells and platelets are normal.

192b Tuttle A H and Koch B Clinical and Hematological Manifestations of Hemoglobin CS Disease in Children J Pediat 56 331 1960

193 Wade L J and Stevenson J D Necrosis of the Bone Marrow With Fat Embolism in Sickle Cell Anemia Am J Path 17 47 1941

194 Watson J Stahman A W and Bilello F I The Significance of the Paucity of Sickle Cells in Newborn Negro Infants Am J M Sc 215 419 1948

195 Watson R J Hereditary Anemias Bull New York Acad Med 30 106 1954

196 Watson R J Hemoglobins and Disease In Snapper I and Dock, W (editors) Advances in Internal Medicine vol 8 Chicago 1956 Year Book Publishers Inc p 305

197 Watson R J Lichtman H C and Shapiro H D Splenomegaly in Sickle Cell Anemia Am J Med 20 196 1956

198 Weens H S Cholelithiasis in Sickle Cell Anemia Ann Int Med 22 182 1945

199 Weissman R Jr Hurley T H Harris J W and Ham T H Studies on the Function of Spleen in the Hemolysis of Red Cells in Hereditary Spherocytosis and Sickle Cell Disorders J Lab & Clin Med 42 965 1954

200 Wells I C and Itano H A Ratio of Sickle Cell Anemia Hemoglobin to Normal Hemoglobin in Sicklemics J Biol Chem 188 65 1951

201 Whipple G H and Bradford W L Mediterranean Disease Thalassemia J Pediat 9 279 1936

202 White J C and Beaven G H A Review of the Varieties of Human Haemoglobin in Health and Disease J Clin Path 7 175 1954

203 Winsor T and Burch G E Habitus of Patients With Active Sickle Cell Anemia of Long Duration Arch Int Med 76 47 1945

204 Wolff J A Michaels R H and Von Hofe F H Hemoglobin H Thalassemia Disease Blood 8 492 1958

205 Zarafonetis C J D McMaster J D Moltham L and Steiger W A Apparent Renal Defect in Sicklemic Individuals Am J M Sc 232 76 1956

206 Zannos L Studies on the Resistance of Haemoglobin to Alkali Acta paediat 42 305 1953

207 Zuelzer W W and Kaplan E Thalassemia Hemoglobin C Disease a New Syndrome Presumably Due to the Combination of the Genes for Thalassemia and Hemoglobin C Blood 9 1047 1954

208 Zuelzer W W Neel J V and Robinson A R Abnormal Hemoglobins In Tocantins L M (editor) Progress in Hematology vol 1 New York 1956 Grune & Stratton Inc p 91

associated with cyanotic congenital heart disease there is no persistence of fetal hemoglobin and in contrast to polycythemia vera the leukocyte and platelet counts are not increased Following operative correction of tetralogy of Fallot reticulocytes are diminished to a very low point and urobilin excretion is greatly increased Both mechanisms a virtual cessation of blood formation and to an even greater extent blood destruction contribute to a reduction of the preoperative polycythemia [1]

Lowered arterial oxygen saturation also prevails in patients with chronic pulmonary disease because of imperfect oxygenation of the blood in the lungs with resulting polycythemia as in patients with emphysema and fibrosis Compensatory polycythemia occurs in persons living in areas at high altitudes where the partial pressure of oxygen is reduced and in those with methemoglobinemia and sulfhemoglobinemia in whom the oxygen-carrying capacity of the red blood cells is compromised by the presence of these pigments The clinical manifestations of chronic mountain sickness which occurs in persons living at high altitudes resemble those of polycythemia [63] Other causes of secondary polycythemia include brain tumors such as cerebellar hemangioblastomas Cushing s syndrome kidney tumors (particularly hypernephroma [0]) hydronephrosis [41] and often cobalt therapy

Abnormally high erythrocyte values in the neonatal period in excess of normal for this period have been described in conjunction with anorexia and nervous system signs and symptoms [6] Polycythemia also may occur as a complication of congenital adrenal hyperplasia[4] in the newborn infant in whom levels of hemoglobin and hematocrit may be so high as to require phlebotomies

Boys and girls from 12 to 18 years of age often normally show maximal hemoglobin values of 16 to 18 gm per 100 ml and red cell counts of 5.5 to 6 5 million per cubic millimeter These peak values simulating polycythemia recede in subsequent years

METHEMOGLOBINEMIA

Iron normally exists in the ferrous state in the iron porphyrin complex of the heme portion of the hemoglobin molecule Methemoglobin is formed when hemoglobin in its deoxygenated state is oxidized to the ferric form In this state iron cannot combine with oxygen and when methemoglobin is produced in significant amounts it reduces the oxygen combining capacity of the blood which is thus incapable of transporting oxygen Methemoglobinemia is characterized by intense cyanosis due to the presence of methemoglobin in the circulating red blood cells in concentrations substantially above normal

Primary or congenital and secondary or drug induced methemoglobinemia are the two major types of this condition In patients with a less common form of congenital methemoglobinemia the defect lies in the globin component and is therefore included in the discussion of abnormal hemoglobins (hemoglobin M) (see Chapter 16)

Pathogenesis Methemoglobin is being formed continuously in the red cells and is simultaneously reduced to hemoglobin by one or more enzymes in the erythrocytes so that the content of methemoglobin within the red cells is less

Primary Polycythemia (Polycythemia Vera, Erythremia, Vaquez Osler Disease)

Primary polycythemia is a specific disease of unknown etiology characterized by cerebral and cardiovascular symptoms a peculiar reddish purple color of the skin bloodshot eyes visual disturbances thrombosis and hemorrhage and usually splenomegaly This disease occurs in middle and old age and is extremely rare in childhood [9,4] The bone marrow is hyperplastic total blood cell formation is increased with a marked elevation of red cells hemoglobin level and hematocrit as well as of leukocytes and platelets The total blood volume is strikingly increased Intravenously administered radioactive phosphorus is the treatment of choice The disease is chronic progressive and ultimately fatal The absence of splenomegaly and particularly normal white cell and platelet counts rule out polycythemia vera

Benign Familial Polycythemia Benign familial polycythemia is another type of primary polycythemia which occurs more frequently in children than does polycythemia vera The patient one or more siblings and in some cases a parent manifest an elevated hemoglobin level (up to 27 gm per 100 ml) red cell count (10 million per cubic millimeter) and hematocrit (82 per cent) without evidence of underlying cardiopulmonary insufficiency There is no splenomegaly or other physical signs except for a ruddy countenance and deeply injected conjunctivae The red cell mass is increased but the leukocyte and platelet counts are normal thus differing from polycythemia vera [30] No treatment is needed the prognosis is favorable

Secondary Polycythemia (Erythrocytosis, Erythrocythemia, Compensatory Polycythemia)

The term secondary polycythemia signifies an increase in the total volume of red cells in response to a recognizable cause Secondary polycythemia is most commonly found in infancy and childhood in patients with cyanotic congenital heart disease due to shunts from the pulmonary circuit The mixture of unsaturated venous blood and arterial blood results in a lowered oxygen saturation The hemoglobin ranges between 15 and 25 gm per 100 ml the red count between 5 5 and 9 million and the hematocrit between 65 and 85 per cent paralleling the red cell counts [1] The mean red cell volume is usually higher than in normal children of corresponding age and weight ranging between 80 and 110 cubic microns [8] The polycythemia serves as an extremely effective compensatory mechanism until the hematocrit reaches levels of 80 per cent or more At this point the disadvantages of coexisting high blood viscosity outweigh the advantages of the increased available oxygen which polycythemia provides [64,1] The response of the bone marrow to the persistent anoxic stimulus which accounts for the polycythemia is probably mediated by a humoral factor [40] It is of interest that a hemoglobin concentration which is regarded as normal for the average child may represent a state of hypochromic anemia in the young patient with cyanotic heart disease and erythrocytosis Iron therapy corrects the hypochromic anemia and is responsible for a further increase in the red cell count hemoglobin level and hematocrit In the patient with polycythemia

disease The absence of signs of heart disease and spectroscopic examination of the blood reveal the diagnosis of methemoglobinemia

Congenital Methemoglobinemia Associated With Hemoglobin M

The defect in congenital methemoglobinemia associated with hemoglobin M lies in the globin component (see Chapter 16) Methemoglobin constitutes 15 to 25 per cent of the total hemoglobin in affected persons They live normal lives and are undisturbed by cyanosis Ascorbic acid and methylene blue exert no effect on the methemoglobin or cyanosis

Drug Induced Methemoglobinemia

Various compounds activate the oxidation of hemoglobin from the ferrous to the ferric state forming methemoglobin These direct oxidants include nitrites chlorates and quinones [6] and prominent among these compounds are aniline and its derivatives sulfanilamides acetanilid phenacetin bismuth subnitrate and potassium chlorate In infancy and childhood the offenders are marking ink shoe dyes certain red wax crayons [69] well water containing nitrates from the soil meat containing a high nitrate content [66] and furniture polish containing nitrobenzene [60] Nitrate from contaminated water from shallow wells used in infant feeding mixtures is converted to nitrite in the bowel and on absorption causes methemoglobinemia [1 34 3] The absorption of aniline dye from marking ink on diapers and other articles of infants clothing has been responsible for outbreaks of methemoglobinemia in nurseries which frequently affect premature infants [31 49 3 67] Similar poisoning from aniline dyes results from cutaneous absorption from freshly dyed shoes and blankets Methemoglobinemia has also been produced in young infants by the application of ointments containing benzocaine [84] Intracorpuscular methemoglobin and sulfhemoglobin have been noted in the course of hemolytic anemia caused by large doses of sulfonamides and toxic agents Heinz bodies and contracted red cells have been noted in the blood smears of affected patients

Features Common to Methemoglobinemia

Diagnosis Methemoglobinemia is suspected when there is a definite history of ingestion of or exposure to a toxic substance when cyanosis exists in the absence of evidence of cardiac or respiratory dysfunction The diagnosis is supported if a sample of venous blood retains the characteristic chocolate brown color after vigorous shaking with air for fifteen minutes Methemoglobin has a well defined absorption band spectroscopically at 630 mμ which disappears on the addition of 5 per cent solution of potassium cyanide A striking feature is the intensive peculiarly grayish cyanosis which develops within one to two hours after ingestion of the toxic substance The discoloration progresses rapidly until the skin and mucous membranes become almost black in color [6] In mild cases the child is fully conscious and in no distress In severe cases the patient has considerable anoxemia and dyspnea and develops circulatory failure

Treatment Patients with mild cases of acquired methemoglobinemia recover spontaneously upon withdrawal of the toxic substance In a patient with disease

than 2 per cent of the total hemoglobin [6] The mean value for methemoglobin has been given as 2 2 per cent in premature infants between 1 and 1 5 per cent in newborn infants and infants in the first year of life and below 1 per cent there after [6] The enzyme system responsible for normally reducing methemoglobin to hemoglobin is dependent upon the integrity of the erythrocyte and is associated with the glycolytic process [6] The effects of the formation of methemoglobin are twofold First methemoglobin as such becomes unavailable for transport of oxygen Second the presence of methemoglobin renders the dissociation curve of the residual oxyhemoglobin less S shaped and more hyperbolic Indeed this change causes a shift of the dissociation curve of the residual oxyhemoglobin to the left The total effect is a lowered capacity for unloading oxygen to the tissues and hence a tissue susceptibility to anoxia [6, 4]

Relatively small amounts of methemoglobin approximating 15 per cent of the total hemoglobin are sufficient to produce cyanosis The relative capacity of the pigments to produce cyanosis of comparable intensity has been given as 5 gm of reduced hemoglobin per 100 ml of blood 1 5 gm per cent of methemo globin and less than 0 5 per cent of sulfhemoglobin [37]

Cardiovascular mechanisms for increasing the oxygen supply to the tissues are called into play when the concentration of methemoglobin exceeds 40 per cent of the blood pigments At levels exceeding 60 per cent ataxia prostration and unconsciousness occur Failure of an oxygen supply necessary for sustaining life could be expected to result when the methemoglobin concentration reaches about 85 per cent [6]

Methemoglobinemia results clinically either from an inborn error of metab olism in which the intracellular enzymes normally reducing methemoglobin are deficient or from the effect of chemical or therapeutic agents which oxidize hemoglobin at a rate beyond the capacity for its reduction

Congenital (Familial) Methemoglobinemia

Congenital (familial) methemoglobinemia is caused by a congenital absence of a reducing factor in the erythrocytes which is responsible for the reconversion of methemoglobin to hemoglobin in normal red cells The defect is often familial [8] transmitted as a recessive Patients with this disease suffer from a deficiency of the flavoprotein coenzyme factor 1 (diaphorase 1) which acts as a carrier in the conversion of methemoglobin to hemoglobin [44] This enzyme deficiency re sults in an accumulation of methemoglobin in the red cells The shift to the left of the oxygen dissociation curve is not always observed [10] Without treatment the majority of these patients tend to reach equilibrium at about 40 per cent methemo globin in the blood Most patients show persistent diffuse cyanosis which is generalized but particularly marked in the fingers toes buccal mucous mem branes lips nose cheeks and conjunctivae Clubbing of the fingers does not occur There is no anemia but mild compensatory polycythemia may develop Patients are usually asymptomatic except for occasional headaches and in older persons poor exercise tolerance may be noted Usually there is no physical dis ability even with strenuous exercise Life expectancy is unaffected Congenital methemoglobinemia may be confused initially with cyanotic congenital heart

Cyanosis due to sulfhemoglobinemia which was both congenital and familial has been described in a newborn infant[6] In another case cyanosis due to sulfhemoglobinemia occurred in a 5 year old child with nonspecific gastroenteritis to whom a triple sulfonilamide had been given in the course of prolonged administration of a sulfur tonic[39]

MISCELLANEOUS ANEMIAS

Anemia of Chronic Renal Insufficiency

Anemia is a well known accompaniment of chronic renal failure irrespective of the type of lesion causing it It occurs in patients with primary diseases of the kidney such as acute and chronic glomerulonephritis chronic pyelonephritis and congenital polycystic kidneys or in those with diseases possessing renal components such as lupus erythematosus Within limits the degree of anemia varies with the level of nitrogen retention

Pathogenesis The two main factors concerned with the pathogenesis of chronic renal failure are the depression of erythropoiesis and increased red cell destruction Inadequate marrow response is evident from the poor utilization of intravenous injections of radioactive iron [3] [9] The actual factor responsible for erythroid depression is still unknown but a toxic retention product remains a possibility There are indications also that kidney disease interferes with erythropoiesis by retarding the elaboration or activation of an erythropoietic stimulating factor produced by the normal kidney [48] [9]

Transfusion of the red cells of the patient or of a normal donor into the patient s circulation reveals a shortened life span for both types of cells [1] [3] [9] The most marked hemolysis as judged by shortened red cell survival occurs during the period of increasing uremia and azotemia It is assumed that rapidly progressive anemia in the absence of detectable blood loss is caused by an extracorpuscular hemolytic factor In patients with stationary chronic renal failure normal donor cells show no shortened survival Increased destruction of red cells in patients with chronic disease carries a poor prognosis Erythrocytes from patients with severe renal failure are normal as measured by their normal life span when injected into healthy recipients [9] indicating the absence of an intracorpuscular defect It seems likely therefore that a combination of depressed erythropoiesis and increased cell destruction is manifest in some stage of chronic nephritis Blood loss from epistaxis and renal and gastrointestinal bleeding may contribute to the anemia since a hemorrhagic tendency is not uncommon in persons with chronic nephritis

Laboratory Findings The anemia is usually normochromic and normocytic occasionally it is microcytic or macrocytic Contracted and deformed erythrocytes assuming a triangular shape have also been observed[77] Reticulocytes may be reduced in numbers[11] or slightly to moderately increased [9] the levels probably depending upon the degree of associated hemolysis Anemia occurs in practically all patients with any significant degree of azotemia [11] Hemoglobin levels average 8 to 9 gm per 100 ml in patients with moderate azotemia[11] but drop to 4 and 5 gm as nitrogen retention increases The white blood cells may be normal in number or slightly increased and there is little or no shift

of any degree of severity treatment should be initiated immediately Methylene blue acts as a specific antidote converting methemoglobin to hemoglobin The recommended dosage for infants is 2 mg per kilogram of body weight for older children 1 5 mg per kilogram and for adults 1 mg per kilogram It is readily available in ampules of 1 per cent solution the solution being given by slow intravenous injection Treatment may be repeated if methemoglobinemia recurs Ascorbic acid orally or parenterally also reduces methemoglobin but the conversion takes place more slowly and therefore is not practical in urgent cases

In patients with hereditary methemoglobinemia orally administered methylene blue or ascorbic acid may be given over prolonged periods to combat cyanosis and particularly associated *symptoms such as headache Ascorbic acid in an oral* dosage of 500 mg given to a 12½ year old boy caused a significant drop in methemoglobin concentration [8] Intravenous methylene blue acts more promptly but a gradual return to the original methemoglobinemia is inevitable when therapy is terminated

Methemoglobinemia in Young Infants

The susceptibility of young infants to the development of methemoglobinemia upon *exposure to certain toxic agents has been shown to be due to a deficient* reduction of methemoglobin in the red cells [69] Well water containing a high percentage of nitrates causes methemoglobinemia in formula fed infants even though their parents drinking the same water remain normal [7, 69]

The differences in the reactions of the infants and their parents are explained by observations that the erythrocytes of cord blood reduce significantly less methemoglobin than do those of adult controls in the presence of lactate lactate and methylene blue or glucose The normal in vivo pathway for reduction of methemoglobin has been shown to be dependent upon the generation of reduced diphosphopyridine nucleotide (DPNH) [44] The red cells of the young infant have difficulty in reducing hemoglobin presumably on the basis of a transient deficiency of either DPNH dependent hemoglobin reductase or of one of the enzymes responsible for its generation [70]

SULFHEMOGLOBINEMIA

Sulfhemoglobin is a pigment not normally present in the body It is inert as an oxygen carrier The combination of inorganic sulfides with hemoglobin in vivo results in its formation [37] Sulfhemoglobinemia often accompanies methemoglobinemia Once sulfhemoglobin is formed it is stable and irreversible disappearing after three to four months when the affected red cells are destroyed In contrast to methemoglobin methylene blue and ascorbic acid are of no value in converting sulfhemoglobin to hemoglobin The absorption band of sulfhemoglobin is at 618 mμ and is unaltered by the addition of potassium cyanide solution

Enterogenous cyanosis formerly of great interest refers to an ill defined clinical syndrome characterized by attacks of cyanosis headache abdominal pain and bowel dysfunction (either diarrhea or constipation) Sulfhemoglobinemia is frequently present often associated with methemoglobinemia In constipated patients *sulfhemoglobinemia* is attributed to the absorption of an enterogenous oxidizing agent such as hydrogen sulfide

dences of intense blood destruction Acute hemolytic anemias occur also in patients with selected viral infections such as virus pneumonia, infectious mononucleosis influenza, and Coxsackie virus A infections[?] In the large majority of patients however overt hemolysis rarely accompanies an acute infection and when it does occur suggests unusual susceptibility of the patient[?] Slight degrees of increased blood destruction insufficient to give clinical manifestations are a more common occurrence Aplastic anemia has also been known to occur in infants and childern with severe infections

Anemia due to chronic infections may be associated with a variety of disease conditions such as pneumonia empyema tuberculosis rheumatic fever osteomyelitis pyelonephritis wound infections brucellosis subacute bacterial endocarditis and intestinal parasites Mild infections do not provoke anemia unless they are prolonged or recurrent As a rule infections of less than a month's duration manifest no significant anemia [1] Once anemia develops it gradually increases in severity with the progression of infection but eventually stabilizes

Pathogenesis Although the mechanism of the anemia of infection has not been completely elucidated there is convincing evidence that toxic depression of erythropoiesis and shortened survival of the red blood cells contribute to its pathogenesis

It will be remembered that the heme portion of hemoglobin is a metal complex consisting of iron in the center of a porphyrin structure The underlying porphyrin of the hemoglobin molecule is termed protoporphyrin Coproporphyrin uroporphyrin, and protoporphyrin represent the most important naturally occurring porphyrin Coproporphyrin is usually the predominant porphyrin in the urine and feces Human erythrocytes contain free coproporphyrin as well as free protoporphyrin In patients with the anemia of chronic infection an excess of free protoporphyrin and coproporphyrin in the red cells and urinary coproporphyrin may be interpreted as an inability of the heme portion of the hemoglobin molecule to utilize synthesized porphyrins

The decreased rate of the erythrocyte production is due to a quantitative defect in the conversion of protoporphyrin into hemoglobin The increased amounts of free coproporphyrin and protoporphyrin in the red cells of patients with anemia of chronic infection are in agreement with this concept The anemia of infection has also been regarded as a disturbance of iron as well as of porphyrin metabolism In patients with infectious states iron is absorbed normally and leaves the plasma rapidly Iron administered intravenously also increases the serum iron transiently being diverted to the tissues and thus rendered not readily available for hemoglobin formation [9]

In addition to depression of erythropoiesis a hemolytic factor has been demonstrated as shown by a shortened red cell life span in patients with a variety of chronic infectious disorders [11] A decreased red cell survival has been noted in children with acute rheumatic fever [64] The fact that the bone marrow is unable to compensate for the moderate increase in the red cell destruction is further evidence that erythropoiesis is depressed [1]

Blood Findings and Other Laboratory Data The anemia due to chronic infection is rarely severe The hemoglobin usually ranges between 8 and 11 gm per 100 ml with a corresponding reduction in the number of red cells

to the left of the oxygen dissociation curve. Platelets are present in normal numbers.

Bone Marrow. The bone marrow is normal or moderately hypercellular in the majority of patients but becomes mildly hyperplastic with excessive nitrogen retention.[11] In a series of thirty patients with uremia Gasser[1] found no relation between the degree of azotemia and the percentage of erythroblasts in the marrow. Both hypoplasia and hyperplasia of erythroid elements coexisted with all grades of azotemia. In five children with renal disease in whom erythroid elements of the bone marrow numbered less than 1 per cent, four showed congenital malformations of the kidney. The remaining patient suffered from the acute hemolytic uremic syndrome[44] consisting of sudden severe hemolytic anemia (hemoglobin 2.6 gm per 100 ml), a positive Coombs test, renal failure (nonprotein nitrogen 137 to 222 mg per 100 ml), thrombocytopenia, hemorrhage and convulsions. Despite the clinical similarity to thrombotic thrombocytopenic purpura, there was no microscopic evidence of this disease. Bilateral renal cortical necrosis was found at postmortem examination.

Diagnosis. Chronic renal failure is to be considered in any child with prolonged and undiagnosed normochromic and normocytic anemia, especially when there is no response to iron therapy. Earlier in the course of the disease the urinary abnormalities may be minimal and easily overlooked and the blood pressure readings may be equivocal. In one such patient the sole anatomic defect was the presence of medullary cysts in otherwise normal sized kidneys.[4] Anemia and a rise in nitrogenous products will also be found in patients with congestive heart failure and impaired renal function. Temporary depression of renal function, azotemia and anemia also follow severe hemorrhage from the stomach and duodenum.[6]

Treatment. Since the severity of the anemia depends upon the degree and duration of nitrogen retention, there can be no lasting improvement with any type of treatment unless renal function is improved. It does not respond to oral or parenteral iron, liver extract, folic acid or vitamin B_{12}. Transfusion with packed cells remains the treatment of choice for symptomatic relief. Fresh blood is preferable because of a possible coexisting hemolytic element, especially in the patient with advanced disease. The bone marrow may compensate to the point of stabilizing the hemoglobin so that transfusions are no longer necessary. *Oral cobaltous chloride has been advocated in the treatment of anemia of chronic renal disease.*[40] Although oral cobaltous chloride therapy temporarily increases erythropoiesis and hemoglobin synthesis, serious toxic complications have prevented its widespread clinical use.

Anemia of Infection

With the advent of antibiotics and other modern therapeutic measures, anemia due to infection has become relatively infrequent. Its incidence, though small, usually involves persons with chronic rather than acute infections. Severe anemias coexisting with infection are usually hemolytic in nature and have been observed in connection with sepsis due to streptococci, staphylococci and pneumococci. Sepsis in the newborn period, for instance, may provoke an anemia with evi

an increase in the number of platelets The anemia is normochromic and normocytic

The number of reticulocytes increase on the second to the third day reaching maximal levels from the fifth to seventh day and terminating by ten to fourteen days The persistence of reticulocytosis is indicative of continued bleeding Polychromatophilia and normoblasts appear with severe hemorrhage During active regeneration there is a tendency toward macrocytosis The increase in hemoglobin and red cells is often followed by a lag in hemoglobin synthesis due to a deficiency of building materials existing prior to the hemorrhagic episode Blood production is proportional to the amount of iron available in the stores or after iron ingestion The intensity of the stimulus is inversely proportional to the anemia depending upon normal bone marrow capacity [19] In the absence of adequate iron stores the anemia becomes hypochromic and microcytic Under favorable conditions and depending upon the size of the blood loss normal values are reached in four to eight weeks The bone marrow shows a normoblastic hyperplasia during the regenerative period Alimentary azotemia occurs in patients with massive hemorrhage in the gastrointestinal tract frequently from a bleeding peptic ulcer [18] The elevation of blood urea nitrogen is due to the absorption of digested hemoglobin

Clinical Features Unless blood loss is rapid and extensive there are few symptoms With significant hemorrhage clinical signs are related to the fall in blood pressure and blood volume Pallor faintness cold sweating restlessness tachycardia rapid breathing and shock are characteristic In the newborn infant posthemorrhagic anemia with a rapid fall in hemoglobin pallor weakness loss of muscle tone and limpness are pronounced Mild jaundice is noted when blood is sequestered in tissues and body cavities as in the patient with a large cephalhematoma

Treatment The immediate steps in treatment are the control of hemorrhage and restoration of blood volume to offset the reduction in blood pressure Transfusions preferably of whole blood are indicated to restore both blood volume and the deficit of hemoglobin and erythrocytes Less blood than the calculated amount lost (see Chapter 7) is given initially to avoid suddenly overtaxing the depleted cardiovascular system Iron is prescribed for at least two months to secure optimal hemoglobin synthesis

Chronic Hemorrhagic Anemia

Etiology Chronic hemorrhagic anemia is due to repeated overt hemorrhages or concealed small hemorrhages frequently from the same sources responsible for a single massive hemorrhage previously described The gastrointestinal tract is most commonly affected with bleeding from ulcerations anomalies such as a Meckel's diverticulum polyps esophogeal varices in portal hypertension at times associated with bleeding from hemorrhoidal vessels bleeding into the the lungs in idiopathic pulmonary hemosiderosis coagulation disorders severe epistaxes idiopathic thrombocytopenic purpura hookworm disease and genitourinary bleeding

Clinical and Laboratory Features Pallor irritability and anorexia occur

The anemia is usually of a normochromic normocytic type but when protracted it tends to become microcytic and hypochromic in which case slight anisocytosis and poikilocytosis are also present Reticulocyte counts are normal or reduced The serum bilirubin is normal and other evidences of blood destruction are lacking despite the shortened life span of the red cells The bone marrow shows no hypoplasia rather the cellularity is normal or increased If the bone marrow is hyperplastic there is an increase in the granulocytic elements and at times of less mature erythroid elements[7] The excessive granulopoiesis of the marrow often is manifested in the younger patient by marked leukocytosis which may reach the intensity of a leukemoid reaction often out of proportion to the nature or degree of infection Hypoferremia is present the serum iron is markedly lowered and the serum iron binding capacity is moderately reduced[13] The serum copper the free erythrocyte protoporphyrin and coproporphyrin and coproporphyrinuria are increased

Clinical Features Pallor is indicative of the anemia but the predominant symptoms and physical signs are those of the basic infection Chronic anemia in children especially when accompanied by an increased sedimentation rate requires a search for an underlying infection The course and prognosis are determined by the nature of the causative infectious process rather than of the anemia

Treatment The anemia is corrected with subsidence of the basic disease and the elimination of the focus of infection Iron therapy is unnecessary for *this substance is abundantly available in the tissues Liver extract vitamin B_{12}* folic acid and other antianemia agents are without value Increased erythropoiesis and hemoglobin synthesis have been reported with the administration of cobaltous chloride but undesirable side effects such as nausea vomiting and goiter limit its usefulness Blood transfusions are given for their supportive effects when the hemoglobin level drops below 8 gm per 100 ml especially in the event of debilitating signs and also when surgical intervention is contemplated

Anemia of Acute Hemorrhage

Etiology Acute blood loss may be primarily external or internal into the *tissues organs or body cavities The severity of the anemia and symptomatology* depend upon the amount and rate of blood loss Anemia may result from trauma hemophilia thrombocytopenic purpura sudden rupture of esophageal varices in portal hypertension and gastrointestinal ulceration in the course of leukemia and in the newborn infant from premature placental separation rupture of a placental blood vessel incision or rupture of the placenta itself and faulty tying or clamping of the umbilical cord

Blood Picture *Immediately following the hemorrhage the blood volume is* reduced and until it is restored the hemoglobin and red blood cell levels may be deceptively high because of vasoconstriction and stasis During the next one to three days when the blood volume is restored by the entrance of tissue fluids into the circulation blood values decrease and give a true indication of the extent of blood loss On the first day there is a transient polymorphonuclear leukocytosis with a shift to the left of these cells the appearance of myelocytes and

covery from anemia was observed in a patient with severe hypochromic anemia and hemochromatosis following the administration of pyridoxine.[76a] The disturbance in pyridoxine metabolism was unassociated with any disturbance in tryptophan metabolism. Intermittent pyridoxine deficiency has also been described in man with recurrent episodes of severe red cell hypochromasia, microcytosis, and a high serum iron.[3] These abnormalities were corrected by the administration of pyridoxine. It is of interest that the hematologic picture in pyridoxine deficiency closely simulates Cooley's anemia.

Vitamin C and the Anemia of Scurvy. In the scorbutic infant the anemia is hypochromic and microcytic; in the adult it is normocytic or slightly macrocytic. Although anemia is a common finding in patients with scurvy, it has not been exactly determined in what manner a lack of vitamin C (ascorbic acid) interferes with hematopoiesis. In Chapter 13 on megaloblastic anemia it is shown that vitamin C plays a part in folic acid metabolism and that its deficiency contributes to the etiology of the anemia. Accelerated blood destruction also has been noted in patients with scurvy.[77] Contributing to the anemia is the loss of blood from hemorrhages characteristic of the disease. Anemia develops in scurvy in spite of adequate supplies of iron, vitamin B_1, and folic acid.[9] The total white blood cell count, differential count, and platelet count are normal. Moderate leukopenia and thrombocytopenia are present in patients with scurvy. Treatment with ascorbic acid corrects the anemia promptly, resulting in an elevated reticulocyte count in four to six days.

Anemia of Hypothyroidism

A mild normocytic or macrocytic normochromic anemia usually accompanies untreated hypothyroidism. The hemoglobin ranges between 8 and 11 gm per 100 ml, and the red cell count ranges between 3.5 and 4.5 million per cubic millimeter. The bone marrow is hypocellular.[8] Fatty bone marrow confined to the long bones has been described in persons with cretinism.[7] The yellow color of the skin in hypothyroidism is due to carotinemia—not to excessive bilirubin. The anemia is refractory to hematinics but responds to thyroid. Hypothyroidism may be superimposed on a pre-existing iron-deficiency anemia, in which case the anemia is hypochromic microcytic and additional treatment with iron is necessary.

Clinical and experimental evidence suggests that the thyroid has a definite influence on hematopoiesis.[5] Removal of the thyroid gland in rabbits results in a moderate and persistent anemia of the macrocytic type.[3] The anemia in persons with hypothyroidism may be regarded as an effect upon the bone marrow of the sluggish oxidation that occurs in all tissues.

Blood Changes in Lead Poisoning

Lead intoxication, except for industrial cases, is relatively common in infants and children, with its highest incidence between the ages of 12 and 36 months.[10] Children are more susceptible to lead intoxication than adults. It is acquired from a variety of sources, such as repeated ingestion of chips of peeling lead paint from walls, plaster, window sills, frames, repainted toys, furniture, and

with markedly lowered hemoglobin and red cell levels. The anemia varies with the duration and intensity of bleeding. The blood picture is typically that of an iron deficiency anemia. As iron stores are depleted a hypochromic microcytic anemia develops. Reticulocytes rarely exceed 5 per cent. Bone marrow activity is slightly increased to the extent of a mild to moderate normoblastic hyperplasia. The white blood count is normal. In patients with persistent anemia examination of the stools for occult blood is mandatory.

Treatment. The source of bleeding must be localized. Iron is prescribed as for patients with nutritional iron deficiency anemia. Transfusions are given only exceptionally to patients in whom greatly lowered blood values have persisted over long periods and preoperatively to those being prepared for surgical eradication of a Meckel's diverticulum or other congenital anomalies.

Vitamin Deficiencies and Anemia

Many of the vitamins, especially those of the B group, are concerned with some phase of erythropoiesis. Although this relationship can be demonstrated experimentally in animals, only deficiencies of vitamin B_1 and folic acid have been definitely found to provoke anemia in man.

Vitamin A. A severe anemia (hemoglobin 6.7 gm. per 100 ml., red cells 2,710,000 per cubic millimeter) was noted in an allergic child whose diet contained no vitamin A supplement. The anemia and other symptoms of vitamin A deficiency promptly responded to large doses of vitamin A.[4]

Riboflavin. A mild anemia has been observed in swine[5] and dogs[6] with riboflavin-deficient diets.

Nicotinic Acid (Niacin). The relationship of nicotinic acid to anemia has not been established. Pellagra is associated with deficiency of nicotinic acid and other nutritional factors. Anemia is inconstantly associated with this disease. It can be microcytic, normocytic or macrocytic[18] and probably results from multiple nutritional deficiencies rather than from nicotinic acid deficiency per se. It is of interest that a patient with hypochromic anemia in the tropics who was refractory to iron therapy responded to nicotinic acid.[30]

Pyridoxine (Vitamin B_6). Nutritional microcytic anemia due to pyridoxine deficiency has been produced in a number of animals, notably in dogs and pigs.[1] The anemia is characterized by irregular reticulocytosis, normoblastemia, polychromatophilia, bone marrow hyperplasia, elevated plasma iron levels, and hemosiderosis in the liver, spleen and bone marrow. The anemia fails to respond to iron or copper but is relieved by synthetic pyridoxine,[14, 83] which also mobilizes the iron deposits from the tissues. Pyridoxine deficiency rarely occurs in man. However, an infant with severe hypochromic microcytic anemia induced by pyridoxine deprivation responded promptly to parenteral and oral administration of the vitamin.[7] Occasionally, anemias refractory to the common antianemia agents have responded to pyridoxine. In one adult refractory hypochromic anemia and abnormalities in tryptophan metabolism were simultaneously corrected by the injection of pyridoxine.[4] In another patient with hypochromic anemia reported on from the tropics in whom there was no diarrhea or steatorrhea a satisfactory response was obtained with pyridoxine.[30] A moderate re-

2 Aub J C Fairhall L T Minot A S and Reznikoff P Lead Poisoning Medicine 4 1 1925
3 Auerbach M L Wolff J A and Mettier S R Benign Familial Polycythemia in Childhood Report of Two Cases Pediatrics 21 54 1958
4 Bass M H and Caplan J Vitamin A Deficiency in Infancy J Pediat 47 690 1955
5 Berk L Burchenal J H and Castle W B Erythropoietic Effect of Cobalt in Patients With and Without Anemia New England J Med 240 754 1949
6 Bodansky O Methemoglobinemia and Methemoglobin Producing Compound Pharmacol Rev 3 144 1951
7 Bomford R Anaemia Myoedema Quart J Med 7 495 1938
8 Breakey V K St G Gibson Q H and Harrison D C Familial Idiopathic Methaemoglobinaemia Lancet I 935 1951
9 Bronte Stewart B The Anaemia of Adult Scurvy Quart J Med 22 309 1953
10 Bush J A Ashenbrucker H Cartwright G E and Wintrobe M M The Anemia of Infection the Kinetics of Iron Metabolism in the Anemia Associated With Infection J Clin Invest 35 89 1956
11 Callen I R and Limarzi L R Blood and Bone Marrow Studies in Renal Disease Am J Clin Path 20 3 1950
12 Cantrow A and Trumper M Lead Poisoning Baltimore 1944 Williams & Wilkins Co
12a Cartwright G E Dietary Factors Concerned in Erythropoiesis Blood 2 111 1947
13 Cartwright G E and Wintrobe M M The Anemia of Infection a Review In Dock W and Snapper I (editors) Advances of Internal Medicine vol 5 Chicago 1952 Year Book Publishers Inc p 165
14 Cartwright G E Wintrobe M M and Humphreys S Studies on Anemia in Swine Due to Pyridoxine Deficiency Together With Data on Phenylhydrazine Anemia J Biol Chem 153 171 1944
15 Chaplin H and Mollison P L Red Cell Life Span in Nephritis and in Hepatic Cirrhosis Clin Sc 12 351 1953
16 Chisholm J J Jr and Harrison H F The Exposure of Children to Lead Pediatrics 18 943 1956
17 Chisholm J J Jr and Harrison H E The Treatment of Acute Lead Encephalopathy in Children Pediatrics 19 2 1957
18 Chunn C F and Harkins H N Alimentary Azotemia Am J M Sc 201 745 1941
19 Coleman D H Stevens A R Jr Dodge H T and Finch C A Rate of Blood Regeneration After Blood Loss A M A Arch Int Med 92 341 1953
20 Conley C L Kowal J and D Antonio J Polycythemia Associated With Renal Tumors Bull Johns Hopkins Hosp 101 63 1957
21 Cornblath M and Hartmann A F Methemoglobinemia in Young Infants J Pediat 33 421 1948
22 Dacie J V The Haemolytic Anaemias Congenital and Acquired New York 1954 Grune & Stratton Inc
23 Dameshek W Stress Erythrocytosis (editorial) Blood 8 282 1953
24 Darling R C and Roughton F J W The Effect of Methemoglobin on the Equilibrium Between Oxygen and Hemoglobin Am J Physiol 137 56 1942
25 Daughaday W H Williams R H and Daland C A The Effect of Endocrinopathies on the Blood Blood 3 1342 1948
26 de Gruchy G C Clinical Haematology in Medical Practice Springfield Ill 1958 Charles C Thomas Publisher
27 Desforges J F and Dawson J P The Anemia of Renal Failure A M A Arch Int Med 101 326 1958
28 Dubach R Moore C V and Minnich V Studies in Iron Transportation and Metabolism Utilization of Intravenously Injected Radioactive Iron for Hemoglobin Synthesis and an Evaluation of the Radioactive Iron Method for Studying Iron Absorption J Lab & Clin Med 31 1201 1946

lead nipple shields and the inhalation of lead fumes from the burning of battery casings. The essential diagnostic features include dense bands at the ends of the long bones and metallic flecks in the large bowel observed on roentgenograms; increased content of lead in the urine (over 0.1 mg. per liter in twenty-four hours) and blood (0.06 gm. per 100 ml.);[17] bluish black and stippled "lead lines" on the gingival margins; increased excretion of coproporphyrin in the urine; history of pica for paint and plaster; and definitive blood changes.

Among the important hematologic changes are basophilic stippling, moderate reticulocytosis, polychromatophilia, microcytic and hypochromic anemia, anisocytosis and poikilocytosis, and an increased number of target cells. The anemia is usually mild, but the hemoglobin level may range from 5 to 10 gm. per 100 ml.[17] Anemia is brought about by the toxic effect of the lead upon hematopoiesis in the marrow and by an increased hemolysis of circulating red blood cells. In the marrow, lead injures nucleated red cells or precursors by interfering with normal maturation, resulting in defective hemoglobinization.[61] The disturbance in hemoglobin synthesis stems from the effect of lead in preventing the incorporation of iron in the protoporphyrin nucleus of heme with resultant excretion of excessive coproporphyrin in the urine (essentially coproporphyrin III) and the accumulation of free protoporphyrin within the red cell.[79] This excess of free protoporphyrin in the erythrocytes accounts for their red fluorescence when thin wet preparations of blood of patients with lead poisoning are examined under ultraviolet light.[98] Several explanations have been given for the accelerated destruction of red cells in the peripheral blood. In vitro studies have shown that lead injures the surface of the red cell, rendering it inelastic, brittle, and susceptible to fragmentation by mechanical trauma, although red cell osmotic fragility is decreased.[9] The increase in stippled cells in the circulation following splenectomy results from the premature elimination of these defective cells by the spleen. The destruction of damaged red cells leads to bone marrow stimulation.

Stippling occurs in patients with many kinds of anemias but may be sufficiently pronounced in those with the mild form of Cooley's anemia to simulate lead poisoning. The basophilic granules, however, are often larger and coarser in persons with lead poisoning than in those with other anemias. The stippled cell has been interpreted as a reticulocyte in which the basophilic material has been altered by lead.[80] Stippling has also been ascribed to the injurious effect of lead on the ribonucleic acid of the young erythrocyte and its precipitation by Wright's stain to give the characteristic punctate basophilic appearance.[9] Many stippled red cells give a positive iron reaction.[61] The bone marrow shows erythroid hyperplasia and stippling of the red cell precursors. No constant changes have been observed in the total white blood cell count or platelet count.[1] Because iron deficiency may antedate or coexist with lead intoxication, iron therapy is required in addition to deleading measures.

REFERENCES

1. Adams, F. H., and Cunningham, S. C.: Further Studies on the Blood of Children With Cyanotic Heart Disease With Special Reference to the Hemoglobin. J. Pediat. 41:424, 1952.

56 Kravitz H Elegant L D Kaiser E and Kagan B M Methemoglobin Values in Premature and Mature Infants and Children A M A J Dis Child 91 1 1956
57 Lawrence J H and Berlin L I Relative Polycythemia—Polycythemia of Stress Yale J Biol & Med 24 498 1952
58 Leonard B J Israels M C G and Wilkinson J F Alkaline Phosphatase in the White Cells in Leukaemia and Leukemoid Reactions Lancet 1 289 1958
59 Loge J P Lange R D and Moore C V Characterization of the Anemia Associated With Chronic Renal Insufficiency Am J Med 24 4 1958
60 MacDonald W P Methaemoglobinaenemia Resulting From Poisoning in Children M J Australia 1 145 1951
61 McFadzean A J S and Davis L J On the Nature and Significance of Stippling in Lead Poisoning With Reference to the Effect of Splenectomy Quart J Med 18 57 1949
62 Miller A A Congenital Sulfhemoglobinemia J Pediat 51 233 1957
63 Monge C Chronic Mountain Sickness Physiol Rev 23 166 1953
64 Nadas A S Pediatric Cardiology Philadelphia 1957 W B Saunders Co
65 Nadler E B and Cohn I Familial Polycythemia Am J M Sc 198 41 1939
66 Orgeron J D Martin J D Caraway C T Martine R M and Hauser G H Methemoglobinemia From Eating Meat With High Nitrite Content Pub Health Rep 72 189 1957
67 Ramsay D H E and Harvey C C Marking Ink Poisoning an Outbreak of Methemoglobin Cyanosis in Newborn Babies Lancet 1 910 1959
68 Reinhold J The Survival of Transfused Red Cells in Acute Rheumatic Fever With Reference to a Latent Haemolytic Mechanism Arch Dis Childhood 29 201 1954
69 Rieders F and Brieger H Mechanism of Poisoning From Wax Crayons J A M A 151 1490 1953
70 Ross J D and Desforges J F Reduction of Methemoglobin by Erythrocytes From Cord Blood Further Evidence for Deficient Enzyme Activity in the Newborn Period Pediatrics 23 718 1959
71 Rudolph A M Nadas A S and Borges W H Hematologic Adjustments to Cyanotic Congenital Heart Disease Pediatrics 11 454 1953
72 Saifi M F and Vaughan J M The Anemia Associated With Infection Brit M J 1 35 1948
73 Sharpe J C and Bisgard J D The Relation of the Thyroid Gland to Hematopoiesis J Lab & Clin Med 21 347 1936
74 Smith C H and Graham J B Congenital Medullary Cysts of the Kidneys With Severe Refractory Anemia Report of a Case Am J Dis Child 69 369 1945
75 Snyderman S E Holt L E Jr Carretero R and Jacobs K Pyridoxine Deficiency in the Human Infant Am J Clin Nutrition 5 200 1953
76 Spector H Maass A R Michaud L Elvehjem C A and Hart E B The Role of Riboflavin in Blood Regeneration J Biol Chem 150 75 1943
76a Verloop M C and Rademaker W Anaemia Due to Pyridoxine Deficiency in Man Brit J Haemat 6 66 1960
77 Vilter R W Woolford R M and Spies T D Severe Scurvy a Clinical and Hematologic Study J Lab & Clin Med 31 609 1946
78 Waisman H A Bain J A Richmond J B and Munsey F A Laboratory and Clinical Studies in Congenital Methemoglobinemia Pediatric 10 293 1952
79 Watson R J Decker E and Lichtman H C Hematologic Studies of Children With Lead Poisoning Pediatrics 21 40 1958
79a Whitaker J A and Vietti T J Fluorescence of the Erythrocytes in Lead Poisoning in Children an Aid to Rapid Diagnosis Pediatrics 24 734 1959
80 Whitby L E H and Britton C J C Relation of Stippled Cell and Polychromatic Cell to Reticulocyte Lancet 1:1173 1933
81 Wintrobe M M Clinical Hematology ed 4 Philadelphia 1956 Lea & Febiger

29 Dystra O H and Hilbertsma T Polycythemia Vera in Childhood Report of a Case With Changes in the Skull Am J Dis Child 60 907 1940
30 Eder H A Finch C and McKee R W Congenital Methemoglobinemia a Clinical and Biochemical Study of a Case J Clin Invest 28 265 1949
31 Editorial Polycythemia Lancet 1 189 1959
32 Emerson C P and Burrows B A The Mechanism of Anemia and Its Influence on Renal Function in Chronic Uremia J Clin Invest 28 779 1949
32a Erslev A J Lear A A and Castle W B Pyridoxine Responsive Anemia New England J Med 262 1209 1960
33 Etteldorf J N Methylene Blue in the Treatment of Methemoglobinemia in Premature Infants Caused by Marking Ink J Pediat 38 24 1951
34 Ewing M C and Mayon White R M Cyanosis in Infancy From Nitrates in Drinking Water Lancet 1 931 1951
35 Faucett R L and Miller H C Methemoglobinemia Occurring in Infants Fed Milk Diluted With Well Water of High Nitrate Content J Pediat 29 593 1946
36 Fichter E G Sulfhemoglobinemia A M A Am J Dis Child 88 749 1954
37 Finch C A Methemoglobinemia and Sulfhemoglobinemia New England J Med 239 470 1948
38 Finch C A Gibson J G II Peacock W C and Fluharty R G Iron Metabolism Utilization of Intravenous Radioactive Iron Blood 4 905 1949
39 Foy H and Kondi A Hypochromic Anemias of the Tropics Associated With Pyridoxine and Nicotinic Acid Deficiency Blood 13 1054 1958
40 Gardner F H The Use of Cobaltous Chloride in the Anemia Associated With Chronic Renal Disease J Lab & Clin Med 41 56 1953
41 Gardner F H and Freymann J G Erythrocythemia (Polycythemia) and hydronephrosis Report of a Case With Radio Iron Studies With Recovery After Nephrectomy New England J Med 259 323 1958
42 Gasser C Aplasia of Erythropoiesis Acute and Chronic Erythroblastopenias or Pure (Red Cell) Aplastic Anaemias in Childhood Pediat Clin North America p 445 May 1957
43 Gasser C Gautier E Steck A Siebenmann R E and Oechslin R Hamolytisch urämische Syndrome bilaterale Nierenrindennekrosen bei akuten erworbenen hamolytischen Anamien Schweiz med Wchnschr 85 905 1955
44 Gibson Q H The Reduction of Methemoglobin in the Red Cells and Studies on the Cause of Idiopathic Methemoglobinemia Biochem J 42 13 1948
45 Gold A P and Michael A F Jr Congenital Adrenal Hyperplasia Associated With Polycythemia Pediatrics 23 727 1959
46 Gordon A S Hemopoietine Physiol Rev 39 1 1959
47 Harris J W Price J M Whittington R M Weisman R Jr Horrigan D L and Cleveland O Pyridoxine Responsive Anemia in the Adult Human J Clin Invest 35 709 1956
48 Harrison T R Principles of Internal Medicine New York 1958 Blakiston Co
49 Howarth B E Epidemic of Aniline Methaemoglobinemia in Newborn Babies Lancet 1 934 1951
50 Jacobson L O Goldwasser E Fried W and Plzak L Role of the Kidney in Erythropoiesis Nature 179 633 1957
51 Josephs H W The Mechanism of the Reduction of Red Cells and Hemoglobin Following Operation for Tetralogy of Fallot Bull Johns Hopkins Hosp 86 1 1950
52 Joske R C McAlister J M and Prankerd T A J Isotope Investigations of Red Cell Production and Destruction in Chronic Renal Disease Clin Sc 15 511 1956
53 Kagan B M Mirman B Calvin J and Lundeen E Cyanosis in Premature Infants Due to Aniline Dye Intoxication J Pediat 34 574 1949
54 Kellman I Primary Polycythemic Manifestations Am J Dis Child 58 146 1939
55 Krammer A Cartwright G E and Wintrobe M M The Anemia of Infection Studies on Free Erythrocyte Coproporhyrin and Protoporphyrin Blood 9:183 1954

CHAPTER 18

Leukocytes—Cell Types

The origin of white blood cells has been described elsewhere in conjunction with blood formation in the embryo (Chapter 1) These cells differ from the erthrocytes in the absence of hemoglobin and in the possession of a nucleus They are larger than erythrocytes ranging from 8 microns for the small lymphocytes to 15 microns for the largest monocytes These colorless corpuscles constitute the most important elements in the body s defenses against invading microorganisms

The leukocytes possess an extensive enzymatic apparatus whose specific uses have not been entirely explored They contain enzymes such as glucuronidase acid and alkaline phosphatase and esterase and biologically important substances of nonenzymatic nature such as glutathione glucuronic acid histamine and glycogen [43]

Growth and Multiplication As in the red blood cells growth and multiplication of the leukocytes are closely identified with the nucleic acid components of the cytoplasm and nucleus Deoxyribonucleic acid (DNA) is found in the chromatin of the cell nucleus and ribonucleic acid (RNA) in the cell cytoplasm and nucleolus These two substances can be differentiated by the cytochemical Feulgen test for deoxyribonucleic acid Following mild hydrolysis with hydrochloric acid and treatment with reduced fuchsin a red color is produced with the dye if deoxyribose is split off [80]

Folic acid (pteroylglutamic acid–PGA) and folinic acid (citrovorum factor) its biologically more active form are indispensable in the synthesis of deoxyribonucleic acid of the cell nucleus In the treatment of patients with leukemia it will be seen that analogues of folic acid and of some of the nucleic acid components such as the purines act as antimetabolites By gaining entrance into the cells they block enzyme systems and interfere with their growth

The functional demands of each of the white blood cells vary in relation to local tissue changes in health and disease The white blood cells require a rich oxygen supply for growth and development in contrast to anoxic conditions required for optimal erythropoiesis [16] When white blood cells are rapidly destroyed as in patients with leukemia with excessively high white cell counts large amounts of uric acid are liberated In patients with acute leukemia being treated

82 Wintrobe M M Buschke W Follis R H Jr and Humphreys S Riboflavin Deficiency in Swine Bull Johns Hopkins Hosp 75 102 1944
83 Wintrobe M M Follis R H Jr Miller M H Stein H J Alcayaga R Humphreys S Suksta A and Cartwright G E Pyridoxine Deficiency in Swine Bull Johns Hopkins Hosp 72 1 1943
84 Wolff J A Methemoglobinemia Due to Benzocaine Pediatrics 20 915 1957
85 Wolman I J Laboratory Applications in Clinical Pediatrics New York 1957 Blakiston Co
86 Wood J L Plethora in the Newborn Infant Associated With Cyanosis and Convulsions a Review of Postnatal Erythropoiesis J Pediat 54 143 1959

plasma In the course of their disintegration cytoplasm which contains gamma globulin is released in the lymph and blood The rate of lymphocyte dissolution has been attributed to hormonal control—more specifically through the medium of pituitary adrenocortical secretions [0 1]

Plasma cells like lymphocytes to which they are related are also associated with antibody formation Evidences of this function are the increased numbers of plasma cells in persons with hyperglobulinemia their deficiency in those with agammaglobulinemia and the high concentration of ribonucleic acid in the cytoplasm Severe impairment of immunologic response and decreased resistance to infection in agammaglobulinemia are associated with a sharp decrease of plasma cells[30]

Erythrophagocytosis Phagocytosis also plays an important role in the removal of damaged or altered erythrocytes from the peripheral blood Polynuclear neutrophils and monocytes which have ingested intact red blood cells occasionally can be observed They have been noted in patients with hemolytic disease of the newborn infant idiopathic acquired hemolytic anemia leukemia promyelocytes[84] chemical poisoning paroxysmal cold hemoglobinuria typhoid fever and other infections In children with acquired hemolytic anemia marked erythrophagocytosis was observed when the buffy coat of the blood had been previously incubated at 37 C for one hour[94] Erythrophagocytosis noted in the peripheral blood and in the spleen of patients with hemolytic anemia has also been related to the opsonic activity of autoantibodies altering the surface of the red cells[6]

L E Phenomenon Phagocytosis is also exemplified by the L E cell which constitutes an important diagnostic feature of disseminated lupus erythematosus which is a febrile disease of unknown etiology characterized by malaise fever skin lesions polyserositis arthralgia cardiac manifestations and renal damage A common feature is a biologically false positive serologic reaction for syphilis [54 58 9] A palpable spleen and enlargement of superficial lymph nodes have been observed in an appreciable number of patients but are inconstant features Of aid in diagnosis is the demonstration of L E cells from preparations of the blood and bone marrow

A normochromic normocytic anemia with a hemolytic component is present in 3 to 5 per cent of patients Leukopenia and an increased number of non segmented neutrophils are seen in about one third of the patients at initial examination and increase subsequently to about one half at some time in the course of the disease[60] Platelets may be reduced to the degree simulating thrombocytopenic purpura With present methods of treatment including ACTH and steroids patients are kept free of symptoms for a considerable length of time Progressive renal failure constitutes the major problem

An important diagnostic test for disseminated lupus erythematosus is the demonstration of the L E cell described by Hargraves[34] The L E phenomenon is an in vitro reaction which depends upon the presence of a nucleolytic factor and active phagocytic cells that are attracted to the lysed nuclear material which it later engulfs

The L E cell is a segmented neutrophil (rarely a monocyte) which has phagocytized an amorphous mass derived most often from the nucleus of poly

with steroids for instance serious disturbances of kidney function may result.

Chemotactic Factors The functional demands for each type of the white blood cells vary with local tissue changes. The stimulus for each type of cell is largely chemotactic. The direction toward which a cell migrates is influenced by the presence of foreign particles infecting organisms or substances elaborated from the site of tissue injury. This property is manifested chiefly by granulocytes, monocytes and eosinophils but not by lymphocytes. The rapid disintegration of cells require a cycle of compensatory and regenerative mechanisms for their replacement. Under pathologic conditions such as pyogenic infections the nucleic acid and other products of tissue destruction serve as a stimulus for proliferation. A leukocytosis promoting factor which stimulates a discharge of granulocytes from the bone marrow has been isolated in inflammatory exudates. Other products produce leukopenia so that the leukocyte level observed at any one time depends upon the interaction of these opposing factors.[43]

The presence of another substance in normal plasma which expels granulocytic leukocytes from the bone marrow into the blood and to some of the organs has been described. This substance represents the expulsion factor which mediates the delivery of polymorphonuclear leukocytes to the circulation to replace those destroyed.[81]

Particularly in infants the bone marrow may produce sufficiently large numbers of myeloid cells to constitute a leukemoid blood picture in the course of a severe infection. In less severe infections in the younger patients abnormal responses may also occur producing a leukocytosis out of proportion to the stimulus.

The stimulus for production of a particular leukocyte varies with each type of cell and depends upon the nature of the chemical substances liberated from the affected tissues. Certain infections stimulate production of polymorphonuclear leukocytes whereas others may depress them.

Functions—Phagocytosis and Antibody Formation The polymorphonuclear leukocytes, monocytes and other reticuloendothelial cells contribute to the body defenses by their motility and their capacity for ingesting and destroying invading bacteria and discharging granulocytes from the bone marrow.[49] Most of the white blood cells are capable of ameboid movement and obey chemotactic stimuli. They play a part in the defense of the body by phagocytic and immunologic means. The ingestion of a particle by living cells is termed phagocytosis. Ameboid mobility of the granulocytes and monocytes may be quantitatively evaluated and serves as an index of their viability.[4] Neonatal leukocytes obtained from cord blood have been found to have lower ameboid and phagocytic activities when compared to maternal leukocytes and those of other adults.[48] This inferiority would indicate an inherent functional difference corresponding to that found in other organs and systems. The relationship to immunity in the neonatal period has still to be determined.

Macrophages refer to the monocytic cells which ingest not only bacteria but also large particles such as red blood cells. Lymphocytes possess slight phagocytic powers but participate more actively in the elaboration of antibodies.[3] These immune substances are associated with the gamma globulin of blood

factor of the patient's plasma There is evidence that the L E factor is a gamma globulin which acts as an antibody and combines directly with the cell nuclei and nuclear nucleoproteins [3 39]

That an immunologic mechanism may be responsible for the nucleophagocytosis of the L E phenomenon is suggested by the associated evidence of autoimmune hemolytic anemia leukopenia and thrombocytopenia in this disease and by the experimental production in vitro of L E cells

In other observations nucleophagocytosis produced experimentally by mixing antileukocytic serum with leukocytes from the same source resulted in structures resembling L E cells[46] According to this hypothesis the L E plasma factor constitutes an "autoimmune" substance which stimulates antileukocyte antibody In the presence of the L E factor white cells are sensitized and phagocytized by other leukocytes[46] and a high antibody serum would be responsible for both the ultimate development of L E cells and the formation of rosettes[46]

Nucleolysis which is fundamental in the L E phenomenon must be distinguished from the nonspecific nucleophagocytosis An example of the latter is the tart cell This cell is occasionally found in normal bone marrow and in persons with pathologic conditions such as lymphoblastoma multiple myeloma pulmonary infection and metastatic carcinoma[46] The distinguishing feature between the L E cell and the tart cell is the difference in the morphologic appearance of the respective inclusion bodies In the L E cell the inclusion body is amorphous and is characterized by the absence of visible chromatin material In the tart cell the engulfed material can be readily identified as a cell nucleus resembling most often the nucleus of a lymphocyte The L E cell must therefore be differentiated from other cells of nonspecific conditions in which an intact nucleus with a normal chromatin pattern is phagocytized The significance of the tart cell is obscure Hargraves[46] who first described both the L E cell and the tart cell emphasized the fact that the latter is usually a histiocyte whereas the former is almost always a neutrophilic polymorphonuclear leukocyte Furthermore he stated that the inclusion body of the L E cell is usually a homogeneous purple staining mass with no visible chromatin pattern

The engulfment of nuclei and nuclear fragments of other cells is commonly observed in specimens of heparinized bone marrow of normal persons and patients with conditions other than systemic lupus erythematosus

Few positive results have been reported if these criteria are adhered to in patients with conditions other than systemic lupus erythematosus[7] The L E cell however have been reported in patients with penicillin reaction and the possibility of the association of this phenomenon with hypersensitivity has been suggested[46]

L E cells should be sought for in patients with unexplained fever leukopenia thrombocytopenia hemolytic anemia arthritis nephrotic syndrome and uremia in which the etiology is obscure [3] In children protracted polyarthralgia fever and leukopenia should lead to suspicion of disseminated lupus erythematosus and a search for L E cells The characteristic scaly eruption of erythematosus over the bridge of the nose and extending to the malar prominences in a butterfly fashion may not always be conspicuous It may reappear on exposure to sunlight and with the cessation of steroids and corticotropin therapy Occasionally patients with autoimmune (acquired) hemolytic anemia and idiopathic thrombocytopenic purpura may develop overt manifestations of disseminated lupus erythematosus with positive L E tests following splenectomy [11]

L E Test at Tests for several simple methods of demonstrating L E cell and ... without the use of anticoagulant have been devised In each of these the patient ... supplies all necessary elements for producing L E cells the L E factor *nuclear ma*

morphonuclear leukocytes or lymphocytes. Swelling and loss of structure of chromatin material results with the lysed nuclear mass acting as a foreign body. This material is chemotactic and attracts phagocytic cells which engulf it. The cell, usually a neutrophil, contains this nuclear mass and because of its size pushes the nucleus of the phagocytizing cell to one side, thus only partially surrounding the inclusion body.

The ingested nuclear material varies in staining reaction from light pink to dark purple. It is stained by basic dyes and is Feulgen positive, indicating its nuclear origin.

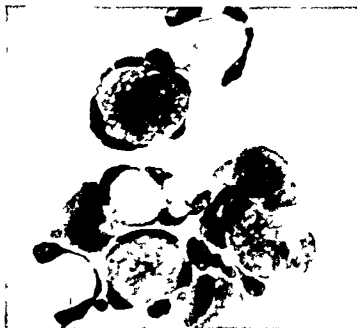


Fig. 29. L.E. cells. Polymorphonuclear cells (usually segmented neutrophils) containing homogenous nuclear material with nucleus displaced to one side. (×1100) (From Holman, Halsted R.: Systemic Lupus Erythematosus. J. Pediat. 56:109, 1960.)

The L.E. rosette results from the attempt of a cluster of leukocytes to phagocytize simultaneously a single large mass of extracellular nuclear material. The presence of a plasma factor is indicated by the development of L.E. cells after incubating the plasma or serum of patients with systemic lupus erythematosus with the buffy layer from bone marrow or peripheral blood of normal persons.

The factor responsible for the L.E. phenomenon in patients with disseminated lupus erythematosus is associated with the gamma globulin fraction of the plasma. There are many explanations for the interaction between L.E. factors and the nucleus of cells. According to one theory, the L.E. plasma factor induces specific chemical changes in leukocytic nuclei (namely, the depolymerization of deoxyribonucleic acid) so that these altered nuclei are later ingested by other leukocytes to form L.E. cells. An alternate theory explains nucleophagocytosis on the basis of antinuclear autoantibodies which correspond to the L.E. serum

Wagner[6] demonstrated leukocyte-destroying factors which were active in vivo and in vitro. Drugs such as Pyramidon combine with a protein in the serum forming an antigen which causes sensitization and antibody formation. The antibody becomes attached to the leukocytes which are agglutinated and destroyed when they come in contact with the antigen.[7] The serum of such a patient not only agglutinates normal human leukocytes in vitro but also produces a rapid fall of the white blood count when it is injected into a normal person. The destruction of agglutinated leukocytes occurs in vivo probably in the lung capillaries.[1]

Agglutination and destruction of circulating leukocytes have a wide application. This process has also been assumed to occur in persons with infections such as primary atypical pneumonia, cyclic agranulocytosis, and pancytopenic states and in newborn infants with transitory granulocytopenia.[44] Bone marrow exhaustion follows peripheral depletion of injured or agglutinated granulocytes.

Leukocyte agglutinins may be detected by adding serum of an affected person to a suspension of leukocytes as completely free of red cells and platelets as possible.[13] The leukoagglutinin may manifest itself as an isoantibody, an autoantibody or an allergic antibody in the patient with drug hypersensitivity. Since the serum substance destroys the patient's own leukocytes, an autoantibody analogous to the antibody against the red cells in patients with acquired hemolytic anemia appears to be involved. Fetal-maternal leukocyte incompatibility and the development of leukoagglutinins have been incriminated in infants with transfusion reactions and neonatal agranulocytosis.

TYPES OF WHITE CELLS

The leukocytes present in the normal blood comprise three main groups: the granulocytic or myeloid series, the lymphocytes, and the monocytes. The bone marrow and lymph nodes give rise to granulocytes and lymphocytes, respectively. Monocytes originate from a reticular cell of the reticuloendothelial tissues present principally in the lymph nodes and spleen and to some extent in the bone marrow and other organs. The individual types of white cells differ from one another in structure and function.

Granulocytic or Myeloid Series

For practical purposes, development of leukocytes may be regarded as proceeding in definitive lines: the myeloblast gives rise to myelocytes and granulocytes, the lymphoblast to lymphocytes, and monoblasts to monocytes. It has already been pointed out that primitive cells of both red and white cell series possess similar structural and staining characteristics and in their maturation reveal many common features. The immature forms of all white cells (the myeloblast, lymphoblast, and monoblast) are not found in the peripheral blood of the normal person, but their differentiation assumes importance in persons with leukemia in whom they infiltrate both bone marrow and blood.

Myeloblast—Differentiation From Lymphoblast. In the patient with the common form of acute leukemia differentiation is usually directed toward dis

terial with which the L E factor can react and the phagocytic cell Thus the use of white blood cells from blood or bone marrow of normal persons is eliminated These tests are carried out with anticoagulants or with the use of clotted blood[80] [0] With the latter caution must be exercised in distinguishing tart cells (damaged nuclear material engulfed by phagocytic cells) from L E cells

Life Span of Leukocytes The life span of the leukocytes has been divided into three phases the hemopoietic phase extending from the development of the primitive cell to its delivery into the circulation the intravascular phase, the period within the circulation and the extravascular phase the period of time the leukocyte spends in the viscera and in the tissues The segment within the circulation therefore must be viewed in relation to the larger extravascular areas The lung liver spleen gastrointestinal tract striated muscle and kidney have been implicated as leukocyte removal sites[4] The pulmonary circulation particularly represents a sizeable reservoir of leukocytes and platelets which may be readily discharged into the circulation under proper stimulation The lungs are optimally located to deliver large quantities of leukocytes and platelets into the circulation more rapidly than are the bone marrow spleen liver or other hemopoietic sites

Various techniques have been used to estimate the life span of the white blood cells but they have not yielded consistent results A recent method[8] utilizes the incorporation of radioactive phosphorus into the deoxyribonucleic acid of the leukocytes The majority of the labeled granulocytes enter the blood stream from the bone marrow at an age of six days and survive in the circulation for about nine days By the same technique two groups of labeled lymphocytes were differentiated—one with a mean age of less than ten days and the majority with a mean age of about 100 to 200 days The life span of eosinophils and basophils is approximately eight to twelve days[6] The life span of three days or less has been given for the granulocyte series in patients with granulocytic leukemia[64] and about thirty days for the lymphocytes in patients with chronic lymphocytic leukemia[c] both obtained by radioactive phosphorus labeling of the white blood cells with subsequent DNA extraction In persons recovering from acute toxic leukopenia approximately fourteen days may be required for maturation of primitive myeloblasts to the four or five lobed polymorphonuclear neutrophil[1] The Pelger Huet anomaly of the granulocytic leukocytes (poor segmentation and condensation of the nuclear chromatin described later on in this chapter) has been employed as a biologic tag to determine the survival time of the transfused neutrophils in the peripheral blood[77] Most of the cells disappeared within six to eight hours and none were found after 49 5 hours These results are comparable to those in a series[4] in which 80 to 85 per cent of the leukemic leukocytes tagged in vivo with a radioactive chromium were removed from the circulation within twenty four hours Some cells persisted for as long as five days

Leukoagglutinins In persons with leukopenic syndromes substances acting upon leukocytes have been detected which are comparable to the antibodies present in the serum of patients with thrombocytopenia and acquired hemolytic anemias In the serum from a patient with agranulocytic angina Moeschlin and

Promyelocytes (Progranulocytes) and Myelocytes Promyelocytes are the same size or larger than myeloblasts A nomenclature committee sponsored by the American Society of Clinical Pathologists and the American Medical Association [7] recommends the term progranulocyte for cells of the granulocytic series which have a nuclear structure too coarse for that of a blast cell and which have not developed discernible specific granules This terminology has not been followed in the present text because it is difficult to identify this cell and because in the bulk of description the cell more mature than the myeloblast is the promyelocyte which does contain granules The cytoplasm is deeply basophilic and may be abundant or confined to a narrow margin around the nucleus The nuclei are round the chromatin is coarser and the nucleoli are not so numerous or so sharply demarcated as those in the myeloblast In contrast to the myeloblast which is devoid of granules promyelocytes and myelocytes are granular In the promyelocyte the granules are relatively few (the earliest forms have no more than ten granules) stain deep red to dark blue and increase in number as the cell matures A few granules overlie the nucleus

The shift from promyelocyte to myelocyte is a gradual one and often difficult to separate into sharply defined categories According to Sabin s classification promyelocytes contain ten granules in early stages and a moderate number in a later stage but the maximal numbers are not present until the myelocyte stage is reached Furthermore the granules in the promyelocyte cannot be differentiated into neutrophils eosinophils or basophils Starting with the promyelocyte and continuing to the most mature granulocytes the granules are oxidase positive and serve as an important feature in differentiating the granulocyte from the myeloblast

Myelocytes are about the same size or smaller than the promyelocyte The nuclei which stain reddish purple are large round oval flattened on one side or somewhat kidney shaped and are eccentrically placed Nucleoli are indistinct and less numerous than those in the more primitive cells Within the reddish purple cytoplasm are numerous dark granules which are scattered throughout and also cover the nucleus As the myelocytes mature the granules assume a definitive neutrophilic eosinophilic and basophilic character Each of these cells in turn becomes a progenitor of the respective fully mature granulocytes

Myelocytes frequently appear in the circulating blood of infants and young children in response to severe infection and hemolytic anemias marked by leukocytosis Because of the more active response of the bone marrow in younger persons the presence of small numbers of late myelocytes usually does not have the same connotation that it has in older persons Occasionally however the leukocytosis and outpouring of young cells in children in response to infections may be sufficiently extreme to constitute a leukemoid reaction

Metamyelocyte or Juvenile Form Metamyelocytes are smaller than the myelocyte and the nucleus is oval and horseshoe shaped in older forms The chromatin strands are coarse but not so deeply stained as those in more mature cells The nuclear membrane is sharply defined as is that in the polymorphonuclear cell and nucleoli are not observed The cytoplasm is less basophilic than that

tinguishing between the myeloblast and the lymphoblast Morphologic characterization however frequently does not permit definite classification into either category Myeloblasts refer to any cell of the granulocytic series having fine chromatin structure and no specific granules Usually nucleoli are visible In patients with some forms of acute leukemia in whom sufficient numbers of promyelocytes justify the classification of the early forms as myeloblasts smaller primitive cells are found which are designated as micromyeloblasts The latter seldom contain nucleoli but they can be distinguished from lymphocytes by their finely meshed chromatin

Lymphoblasts may have a more sharply defined nuclear membrane a coarser chromatin network and a smaller number of nucleoli than myeloblasts have but these features are not always detectable The associated presence of substantial numbers of younger lymphocytes (prolymphocytes) and mature lymphocytes or promyelocytes and later myelocytes suggests a more definitive classification of the predominating immature cells as either lymphoblasts or myeloblasts Both types of blast cells possess certain common fundamental diagnostic features They are usually round large (15 to 20 microns in diameter) and uniform in size The narrow zone of the cytoplasm is basophilic and frequently vacuolated and the nuclear pattern is distinctive Instead of masses of dense basichromatin such as are found in the mature cells the chromatin stains lightly and is finely granular and stippled or sievelike and shows the presence of nucleoli In the absence of cytologic differences the primordial cell is often classified as a stem cell

The morphologic differences between the myeloblast and lymphoblast when stained by Wright s method are listed in Table 14 [7] In acute leukemia of childhood the predominant blast cells usually favor the designation of lymphoblasts Monoblasts are difficult to distinguish from myeloblasts and their diagnosis depends upon the presence of the more mature monocytes resembling them in the abundant granular gray blue cytoplasm and in the characteristic folded and indented nucleus

Table 14 Morphologic Differences Between the Myeloblast and Lymphoblast

	Myeloblast	*Lymphoblast*
Cytoplasm	Abundant	Scanty
Nuclear membrane	Smooth and thin	Dense
Chromatin	Fine network finely divided particles	Coarse and some aggregation
Nucleoli	2 to 5	1 to 2
Nucleolar membrane	Indefinite	Distinct
Mitochondria (supravital staining)	Fine spherical scattered diffusely in cytoplasm	Larger oval thicker than myeloblast frequently clustered about nucleus or scattered through cytoplasm

of the human polymorphonuclear neutrophilic leukocyte has been described.[14] In the female a solitary "drumstick" with a well defined solid round head 1.5 microns in diameter is joined by a single fine chromatin strand to one of the main lobes of the nucleus. These structures are rarely found in unsegmented forms or precursors. This distinctive nuclear appendage which has been confined to the female has been found in persons of all ages and in blood specimens from the umbilical cord.[6] These appendages are not to be confused with sessile nodules and related structures which are attached to the nucleus of the polymorphonuclear cell in both the male and female.

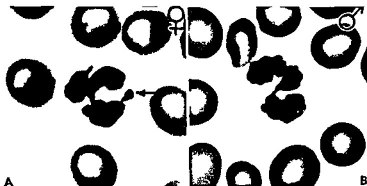


Fig. 30. *A*, Neutrophil in a blood film from a chromosomal female illustrating the accessory nuclear lobule that is present in an average of 2 to 3 per cent of neutrophils in females (×1800). *B*, A similar accessory nuclear lobule does not occur in neutrophils of chromosomal males (×1800). (From Grumbach, M. M., and Barr, M. L.: In Pincus, G.: Recent Progress in Hormone Research, vol. 14. New York, 1958, Academic Press, Inc.)

Eosinophils. The eosinophil is a polymorphonuclear granulocyte the same size or a little larger than the neutrophil and possesses a bilobed or band formed nucleus. The cytoplasmic granules are large, coarse, and spherical, have an affinity for the eosin stain, and take a bright reddish orange or deep red stain. The granules are scattered though the cytoplasm and superimposed on the nucleus. Eosinophils are less motile and more fragile than neutrophils. They represent 1 to 4 per cent of the total number of leukocytes and possess a life span of six days.[4]

EOSINOPHILIA. Eosinophilia occurs in patients with several well defined groups of diseases in which there is an increase in eosinophils to about 5 per cent or 500 per cubic millimeter. Following is a list of conditions in which eosinophilia occurs:

Allergic disorders
- Bronchial asthma
- Hay fever
- Urticaria

Skin diseases
- Eczema
- Psoriasis

in the myelocyte and an eosinophilic cast predominates The granules are smaller stain less deeply and are clearly differentiated as neutrophilic eosinophilic and basophilic Ameboid motion initially observed in the late myelocytes is definitely established in the metamyelocyte and characterizes the more mature cells to follow The cytoplasm and nucleus may not develop evenly so that precise classification as to late myelocyte and metamyelocyte is not always possible

Polymorphonuclear Granulocytes The mature granulocytes are described as polymorphonuclear cells because *of the lobulation of the nucleus and are* further classified as neutrophils eosinophils and basophils according to their reaction with the ordinary Romanowsky stains

Neutrophils The polymorphonuclear neutrophil measures 9 to 12 microns with an average diameter of 10 microns The cytoplasm is faintly pink and minute granules which fill the cell stain pink or violet The nucleus consists of coarsely condensed chromatin strands which stain deep purple

The nucleus is of two types—nonsegmented and segmented according to the absence or presence of lobulation Segmented neutrophils which normally number 60 to 65 per cent of the white blood cells are mature cells in which the nucleus consists of two to five lobes connected by a thin strand of chromatin The nonsegmented cells number 4 to 5 per cent of the total number of white cells and are variously designated as staff or stab cells They are slightly smaller than metamyelocytes in which the nucleus fails to segment presumably due to toxic or other influences Irregular condensation occurs in the nucleus of these cells with a pyknotic area at each end Constriction of the nucleus in the middle or in other areas leads to odd shaped segmented forms In the cells of persons with some infections the nucleus is short narrow bent on itself and deeply stained Granulation in these cells is usually neutrophilic but may be eosinophilic or basophilic

"LEFT AND RIGHT SHIFTS (ARNETH COUNT COOKE AND SCHILLING COUNT) According to Arneth s theory the nucleus of the polymorphonuclear cells becomes more segmented with age The round or oval nucleus of the myelocyte becomes more indented as the cell matures with increasing segmentation in progressively older cells As modified by Cooke and Schilling and in accordance with present usage a *shift to the left" implies the presence in the blood stream of immature* forms consisting of large numbers of band forms This shift is noted in infections toxic states and hemorrhage with the extent of the shift proportional *to the* extent of the disturbance In patients with mongolism without infection there is a shift to the left in lobe count In younger persons severe infections are often accompanied by the additional presence of myelocytes and metamyelocytes The blood smear of patients with pernicious anemia sprue and megaloblastic anemia of infancy on the other hand demonstrate a shift to the right in which older neutrophilic polymorphonuclear cells predominate In these conditions many of the granulocytes may be abnormally large and show hypersegmentation of their nuclei Here too the lobes may vary in size and the chromatin is not so uniformly homogeneous as in normal cells

SEX DIFFERENCE IN NEUTROPHILS A sex difference in the nuclear structure

of the human polymorphonuclear neutrophilic leukocyte has been described[14] In the female a solitary "drumstick" with a well-defined solid round head 1.5 microns in diameter is joined by a single fine chromatin strand to one of the main lobes of the nucleus These structures are rarely found in unsegmented forms or precursors This distinctive nuclear appendage which has been confined to the female has been found in persons of all ages and in blood specimens from the umbilical cord[6] These appendages are not to be confused with sessile nodules and related structures which are attached to the nucleus of the polymorphonuclear cell in both the male and female

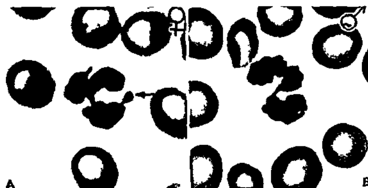


Fig 30 *A* Neutrophil in a blood film from a chromosomal female illustrating the accessory nuclear lobule that is present in an average of 2 to 3 per cent of neutrophils in females (×1800) *B* A similar accessory nuclear lobule does not occur in neutrophils of chromosomal males (×1800) (From Grumbach M M and Barr M L In Pincus G Recent Progress in Hormone Research vol 14 New York 1958 Academic Press Inc)

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- Hay fever
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Skin diseases
- Eczema
- Psoriasis

Skin disease—cont'd
- Scabies
- Erythema neonatorum

Parasitic infections
- Trichinosis
- Ascaris
- Echinococcus disease
- Visceral larva migrans

Pulmonary eosinophilia
- Eosinophilic pneumonitis (Loffler's syndrome)
- Prolonged pulmonary eosinophilia
- Tropical eosinophilia
- Pulmonary eosinophilia with asthma

Blood disorders
- Eosinophilic leukemia
- Hodgkin's disease
- Recovery phase of acute infectious lymphocytosis
- Postsplenectomy

Irradiation

Familial Eosinophilia

Miscellaneous infections and disorders
- Scarlet fever
- Chorea
- Drugs and chemicals
- Benzol poisoning
- Camphor, phosphorus, etc
- Periarteritis nodosa
- Metastatic neoplasms

From the time when it was first demonstrated that foreign materials such as parasitic extracts were chemotactic for eosinophils, the relationship of these cells to diseases of sensitization has been amply confirmed. The number and proportion of eosinophils are greatly increased in patients afflicted with various types of allergy, with parasitic infection, especially by nematodes, and following anaphylactic reactions. The eosinophil is the only cell which is known to respond specifically during states of hypersensitivity, becoming attached to shock tissues.[9] The cell, being phagocytic for foreign substances, is a part of the body defenses.

A recently described condition observed in pediatric practice which is associated with eosinophilia is visceral larva migrans. This entity refers to infection with dog and cat ascarids (*Toxocara canis* and *Toxocara catis*, respectively) and should be considered in the differential diagnosis of febrile illnesses associated with eosinophilia. A history of eating dirt or of exposure to dogs and cats in the home in patients with eosinophilia suggests visceral larva migrans associated with Toxocara infection. It represents an imperfect adaptation of the parasite to the host so that its life cycle in the latter is not completed. The eggs reach the soil through fecal contamination, where within weeks they develop to infective stage larvae. If ingested by a child, the larvae migrate through the intestinal wall via the vascular system and reach the liver, lungs, brain, eye, and other organs. Instead of completing the migration back to the intestine as in the normal dog or cat host, they either become encysted and are destroyed or continue to migrate through the tissues.[8]

Although liver biopsy may reveal characteristic larvae the diagnosis should be considered in the absence of this examination in young exposed patients with persistent or marked eosinophilia recurrent wheezing associated with pulmonary infiltration hepatomegaly of undetermined nature ocular disorders of obscure etiology unexplained central nervous system disturbances including convulsions and hyperglobulinemia [3] In a patient with a similar condition there was an additional finding of a marked and persistent elevation of the heterophil agglutinin titer with antibodies similar to those found in persons with serum sickness [5] Cases previously described as allergic hyperergic tissue responses resulting in disseminated visceral lesions[6] probably fit into this category as well

Moderate eosinophilia with values of about 25 per cent can persist with fluctuations for a period of years in children without any identifiable cause except allergic symptoms A syndrome of extreme leukocytosis and eosinophilia (up to 84 per cent) has been reported by Bass in which children who were chronically affected had a favorable outcome When a normal leukocyte count has been restored a mild eosinophilia may persist In the light of recent studies it is necessary to eliminate visceral larva migrans infection in patients with chronic eosinophilia

Pulmonary involvement accompanied by eosinophilia occurs in a number of distinctive syndromes in which this combination exists Loffler's syndrome the best known of these is a benign condition characterized by a patchy migratory infiltration which usually clears within a month The accompanying eosinophilia reaches its peak in three to four days and disappears usually in ten to fifteen days It is generally regarded as a response to an immune reaction taking place largely in the lungs It has been suggested however that infiltrates in patients with Loffler's syndrome are due to the trapping of the eosinophils in the lung and not to an allergic pulmonary reaction [7] It has been produced by infection with intestinal parasites plant products bacteria and drugs

Prolonged pulmonary eosinophilia another syndrome described by Crofton and associates [8] is to be considered when radiographic shadows and eosinophilia persist for prolonged periods and are more widespread than in patients with Loffler's syndrome It lasts two to six months and longer before recovery sets in and is marked by extreme eosinophilia In many of these patients there is a personal or family history suggestive of an allergic diathesis Probable etiologic factors include hypersensitivity to drugs and bacterial and parasitic infection

Tropical eosinophilia or "eosinophilic lung" occurs in the inhabitants of India and many tropical countries In persons with this syndrome there is sometimes an initial stage of malaise fever coryza and dry cough the spleen may be palpable and eosinophilia is marked The roentgenogram shows a diffuse mottling throughout both lung fields although sometimes the lesions are more localized There may be an allergic background but more recently it has been ascribed to a mite infection of the upper respiratory tract

Patients with pulmonary eosinophilia with asthma includes those in whom pulmonary infiltrations and eosinophilia are associated with symptoms incidental to chronic or recurrent bronchial asthma

Patients with eosinophilic leukemia in contrast to those with other forms of eosinophilia show a marked relative and absolute increase in the circulating mature eosinophils which also infiltrate the tissues during the course of the disease. Patients with this type of leukemia respond for varying periods to ACTH and the steroid hormones.[19] They usually show no pulmonary changes of the magnitude described in patients with other conditions such as pulmonary eosinophilia except when an associated pneumonia occurs. Evans and Nesbitt reported on a patient who typifies others in whom the eosinophils showed a progressive immaturity as symptoms become more severe. Terminally a large proportion of the cells were myeloblasts in both blood and bone marrow. During this period, however, in which the only abnormality is confined to excessive numbers of mature eosinophils, conditions other than leukemia are to be considered.

With the elimination of the major etiologic factors in causation, cases of chronic eosinophilia in which the blood response persists for many years before the blood count eventually returns to normal are still encountered in children with poorly defined allergies.

EOSINOPENIA. Eosinopenia occurs following stress and has been related to the release of adrenocortical hormones. A prompt decrease occurs in circulating eosinophils in healthy persons following a single injection of adrenocorticotropic hormone. The eosinophilic response must be interpreted as a nonspecific stress reaction of the intact organism. Eosinopenia occurs after administration of ACTH, epinephrine, surgical operations, infections, cardiac failure and any other conditions of stress.

The fall in the eosinophil count following injections of adrenocorticotropic hormone (ACTH) has become widely used as a test of adrenal function. In the Thorn test[83] following the preinjection determination of the absolute eosinophil count, 25 mg. of ACTH is given intramuscularly to the child or adult and the percentage decrease is measured in four hours. A reduction of 50 per cent or more in the eosinophil count is presumptive evidence that the cortical tissue has the normal capacity to respond to stimulation. A significant fall in eosinophils therefore does not occur in patients with chronic adrenal insufficiency such as Addison's disease.

No such clear cut results are obtained in the newborn infant. Apparently dosages ranging from 5 to 10 mg. are necessary to provoke eosinophilic responses beyond the range of spontaneous changes.[91] Farquhar[6] found a normal response in the newborn infant with a decrement of 30 per cent or greater when a 10 mg. dose was injected. *On the basis of these observations the presence of physiologic transitory adrenal insufficiency in the newborn period depends upon the standardization of dosage. With adequate dosage the response of the adrenal cortex is found to be normal in the majority of newborn and premature babies in the first days of life.*[91]

Basophils. Basophils originate in the bone marrow and are regular constituents of the peripheral blood. These cells are distinguished by the large, round, coarse, bluish black and azurophilic granules which obscure the nucleus by their number. These granules are peroxidase negative and are situated above, below

and at each side of the relatively lightly staining nucleus The nucleus is round kidney shaped and slightly lobulated The cytoplasm stains faint pink to lilac The basophil is motile and somewhat smaller than the mature neutrophil The basophils number 0 to 0 5 per cent in children and adults All stages of leukocytic differentiation are demonstrable in bone marrow for basophilic white blood cells They are increased in patients with chronic myeloid leukemia polycythemia chronic hemolytic anemia Hodgkin s disease smallpox chickenpox some cases of cirrhosis of the liver after radiation and following splenectomy In patients with lobar pneumonia acute rheumatic fever or anaphylactoid purpura the number of circulating basophils has been found to be low in the acute stage and high during recovery

Tissue Mast Cells (Tissue Basophils) The tissue mast cell is also a granular basophil which shows little motility as compared with the active basophils The separation of the tissue mast cells from the circulating basophils has been a subject of controversy [66 79] Both have in common metachromasia of the granules The cytoplasm of the mast cells is filled with granules which stain well with methylene blue Wright Giemsa stain or toluidine blue (1 per cent toluidine blue in methyl alcohol) Mast cells are found in groups about blood vessels and in the connective tissue where they originate

Tissue mast cells however can be differentiated from blood basophils and are not interchangeable with them Tissue mast cells do not possess myeloid precursors as do basophilic leukocytes Functionally the two cells may have much in common in the production of several biologically active substances especially in the associated content of heparin and histamine in their granules [69] Serotonin (5 hydroxytryptamine) has also been isolated from the tissue mast cells There is suggestive evidence that serotonin and histamine act in conjunction to cause capillary permeability hyperemia and edema which constitute the vascular response to acute inflammation [4]

Morphologically tissue mast cells can be differentiated from basophilic leukocytes The former possesses a normal vesicular nucleus which is rarely indented but the cytoplasmic contours vary widely being round irregularly oval spindle or star shaped The bluish cytoplasm may be hidden because of the heavily packed granules These do not overlie the nucleus as they do in the basophil By contrast the blood basophils usually have a small cell body with a polymorphic or lobulated nucleus typical of leukocytes and the granules tend to be irregularly distributed [8] Accumulations of mast cells are observed in both the macular and papular type of urticaria pigmentosa a dermatologic disorder occurring principally in childhood

Lymphocytes

Lymphocytes originate from lymphocytic tissue in many parts of the body mainly from the lymph nodes spleen bone marrow tonsils thymus gastrointestinal tract and liver Considerable lymphocyte formation takes place in Peyer s patches Lymphocytes enter the blood stream in two ways—indirectly through the thoracic duct right lymph duct or other lymphatic venous communications or directly through the walls of blood capillaries in lymphoid tissue They

are actively motile cells whose function in antibody formation has already been discussed They are classified into large and small cell types with the latter predominating Large and intermediate forms of lymphocytes are assumed to be younger and less mature than small lymphocytes but this premise is to be established

Small Lymphocytes Small lymphocytes vary in size from 6 to 10 microns in diameter usually about the same size as red blood cells They contain a well defined round oval or indented nucleus with closely packed aggregates of chromatin which stains deep purple The nucleus fills almost the entire cell with the periphery sharply defined and more heavily stained The cells have a narrow rim of bluish cytoplasm which is often frayed and stains clear pale blue Although the cytoplasm is usually devoid of granules in well stained preparations some of the cells contain a few distinct reddish purple or azurophilic granules of medium size In thick smears the lymphocytes are spindle shaped with pointed projections of cytoplasm at opposite poles of the cell This feature has no pathologic implications

Large Lymphocytes Large lymphocytes vary from 10 to 15 microns in size The nucleus is larger than that in the small lymphocyte round slightly indented and thickened at the margins and the chromatin material is paler and not so markedly condensed as in the small lymphocyte The cytoplasm is more abundant than in the small lymphocyte and frequently contains azurophilic granules and the edges are frequently scalloped and stain clear light blue A perinuclear clear zone is a differentiating feature

Young Lymphocytes (Prolymphocytes) Prolymphocytes are occasionally found in normal blood and more frequently in the blood of children especially those with chronic upper respiratory infections with associated tonsillar and cervical node enlargement The cell is the same size as the large lymphocyte or is intermediate between the large cell and the small cell The nucleus is less compact with light spaces between the chromatin threads The most important feature is the deep blue basophilic staining of the cytoplasm which is homogeneous and agranular

During the first months of life small numbers of lymphocytes which resemble the abnormal cells found in patients with infectious mononucleosis are occasionally observed in the blood smears from normal infants These cells however bear no relationship to this disease or to any other related condition Scattered vacuoles may be found normally in the rim of deeply staining blue cytoplasm These cells are to be differentiated from the heavily vacuolated cells producing a foamy appearance characterizing the lymphocytes of infectious mononucleosis

Lymphoblasts Lymphoblasts from which the lymphocyte is derived are characterized by a large round or oval nucleus staining reddish purple with fine granular chromatin which is slightly coarser than that found in the nucleus of the myeloblast One or two nucleoli are present in the lymphoblast the nuclear membrane is well defined and the cytoplasm is usually clear blue or deeply basophilic and nongranular

Monocytes

Monocytes measuring from 13 to 20 microns are larger than most cells found in the peripheral blood They represent 5 to 10 per cent of the total circulating white blood cells The monocyte is a motile cell with slow ameboid motion in contrast to the more active neutrophil The cytoplasm is abundant in relation to the nucleus and stains darker than the pink cytoplasm of neutrophils The pseudo pods are blunt The cytoplasm is a dull gray muddy blue color and is filled with large numbers of evenly spread fine lilac or reddish blue granules interspersed among which are a lesser number of unevenly distributed azuro philic granules and occasional vacuoles The granules are peroxidase positive but in much lesser numbers and finer than those in the granulocytes The gray blue cytoplasm of the monocyte contrasts with the clear light blue of the lymphocyte

The nucleus is somewhat eccentric and possesses a skeinlike or lacy structure which stains lighter than that of the lymphocyte or metamyelocyte It is a loosely arranged chromatin with light spaces and grooves in contrast to the clumped chromatin of the lymphocyte The nucleus is indented multilobulated and convoluted and often presents a folded appearance The nuclear folds with the heavier staining at their margins are characteristic of the monocyte The mono cyte phagocytoses red cells cellular fragments and the incompletely lysed nuclei of other cells as occurs in the formation of the tart cell

Young Monocyte (Promonocyte) Although the nucleus of the mature mono-cyte may be round it is more likely to be so in the young monocyte The cell is somewhat larger and the cytoplasm is perhaps less granular and grayer than that in the older cell The features are intermediate between those of the mature cell and the monoblasts

Monoblasts Monoblasts are difficult to differentiate from the corresponding myeloblasts and promyelocytes The cells possessing an irregular cell outline due to the presence of blunt pseudopodia are nonmotile The cytoplasm is deeply basophilic with a grayish blue cast differing in this way from the blasts of other cell series The cytoplasm contains dustlike reddish blue granules and vacuoles The nucleus is large and round often kidney shaped horseshoe shaped or convoluted like the mature cell The chromatin is fine and lacy and stains lighter than that in the mature monocyte Nucleoli may or may not be present The oxidase reaction is negative or poorly defined The presence of monocytes and promonocytes in the same smear facilitates the diagnosis of the primitive monoblasts

The Naegeli and Schilling types of acute monocytic leukemia[89] are de scribed In the Naegeli variety the myeloblast is regarded as the precursor of the monocyte so that the leukemia is myelomonocytic The peripheral blood shows a predominance of monocytes in various stages of maturity in association with myeloblasts and young myelocytes Many of the monocytic precursors may be difficult to distinguish from myelocytes In the Naegeli type the monocytic cell also has a markedly distorted nucleus with a polymorphous shape the entire cell is extremely large and the cytoplasm which is abundant is filled with fine purplish red granules smaller than the larger azure granules of the

myelocytes Scattered in the cytoplasm are small numbers of azure granules similar to those observed in the myelocyte

The Schilling type *is a true monocytic leukemia in which the reticuloendo thelial* cells have been transformed into monocytes In this type the cells range from mature monocytes to monoblasts with only an occasional myelocyte Many of the primordial cells are difficult to differentiate from lymphoblasts or myelo blasts but the majority possess the characteristics of monoblasts In some of these cells and particularly in the promonocytes the nucleus is semitransparent lacy and folded on itself and the deeper underlying folds are clearly visible Promyelocytes and monocytes show a variable reaction with the peroxidase stain some negative and other weakly positive The Schilling type is often re garded as a variant of leukemic reticuloendotheliosis It is the less common of the two types of monocytic leukemia

Monocytosis Monocytosis occurs in the recovery phase of acute infection during the resolution process of pneumonia in the acute phase of rheumatic fever in active tuberculosis in Boeck s sarcoidosis in Hodgkin s disease in pro tozoal infections such as malaria in the recovery phase of agranulocytosis and in disorders of lipid metabolism A moderate increase in young monocytes may accompany the outpouring of the abnormal lymphocytes which characterize the blood picture in infectious mononucleosis Increased numbers of monocytes often occur in children with mesenteric adenitis accompanying upper respiratory infections in contrast to the polynuclear leukocytosis observed in those with appendicitis

Monocytes in Tuberculosis—Monocyte Lymphocyte Ratio Sabin and co work ers[9] demonstrated that the major effect of the tubercle bacillus was on the mono cyte of the connective tissues and blood The epithelioid cell of the tubercle has been proved to be an altered phase in the life cycle of the monocyte Ac cording to this view the lipoids of the tubercle bacillus are solely responsible for the monocyte epithelioid proliferation and differentiation in patients with tuberculosis[9,1] Whereas the tubercle bacillus grows and multiplies within the monocyte lymphocytes are intimately connected with limiting the spread of the infection In patients with tuberculosis the ratio of monocytes to lymphocytes in absolute numbers has been used to express the extent of invasion as compared to the degree of resistance An increase in monocytes with an increase of the ratio of monocytes to lymphocytes indicates a serious prognostic sign a lowering of the ratio with an increase in lymphocytes indicates a favorable sign[8]

Others have observed that the changes in the monocyte lymphocyte ratio are caused more often by a consistent decrease in lymphocytes during active disease and increase during healing than by the more irregular fluctuations of the mono cytes According to this view a sounder interpretation of the *clinical status of* the tuberculosis process can be made by evaluating each cell type separately especially the lymphocyte than by calculating the monocyte lymphocyte ratio[3]

Miscellaneous

Histiocytes Histiocytes are occasionally seen in the peripheral blood and in the bone marrow They are derivatives of the reticuloendothelial system and are

DEGENERATIVE AND TOXIC CYTOPLASMIC CHANGES

Amato Bodies Amato bodies are irregular pale blue staining cytoplasmic clumps up to 2 microns in size They occur in polymorphonuclear neutrophils in patients with various infections such as diphtheria scarlet fever pneumonia, and other coccal conditions and have been mistaken for inclusion bodies possibly of etiologic significance Amato bodies are probably the same as Dohle bodies (see p 338)

Toxic Granules Toxic granules and vacuolization are also regarded as degenerative changes occurring in the cytoplasm during acute infections Excess vacuolization may be especially noticeable in patients with severe infections and in the blast cells of patients with leukemia The toxic granules are deeply staining irregularly distributed coarse basophilic granules that are oxidase negative and occur in segmented and nonsegmented polymorphonuclear neutrophils They have been shown to be liquid droplets and not granules and represent a leukocyte that has undergone cloudy swelling [33]

Pelger Huet Phenomenon of Granulocytes Pelger Huet phenomenon of granulocytes is a hereditary anomaly characterized by partial or complete failure of segmentation of polymorphonuclear cells so that the number of nonsegmented cells is enormously increased The nucleus is usually broad and kidney shaped and sharply delimited consisting of strands of intensely stained chromatin with the intervening spaces clear The conglomerates are particularly prominent in the blunt end of the nucleus Segmented cells with two and sometimes three lobes occur less frequently but the clumping and condensation of chromatin are present in these cells as well The presence of the nonsegmented cells here does not represent a shift to the left as occurs in patients with infections and usually has no effect on health This anomaly is transmitted as a non sex linked mendelian dominant character [3 5] An acquired form has been described in an adult with chronic myelogenous leukemia [1] The Pelger Huet anomaly has also been described as an acquired form in other myelopathies such as acute leukemia and myeloid metaplasia especially following prolonged exposure to myelotoxic therapeutic agents [19a]

Russell Bodies The cytoplasm of a plasma cell may contain prominent spherical hyaline bodies or globules which take an acidophilic stain These masses termed Russell bodies occur singly or in groups and are regarded as consisting of mucoprotein secreted by the parent cell They appear in the plasma cell in myeloma and in plasma cell leukemia [6]

Hematogones Lymphocyte like cells termed hematogones regarded as blood cell precursors are occasionally encountered in the bone marrow rarely in the *peripheral blood In contrast to the large erythrogones (hemocytoblast) these* cells resemble small lymphocytes in size and morphology They differ from the lymphocytes to the extent that they possess a denser more homogeneous slightly purplish dull matlike nucleus either with a narrow rim of cytoplasm or entirely devoid of it Occasionally the nucleus is partially or completely traversed by a fine cleft The hematogones often present the appearance of naked nuclei and also differ from the nucleus of the normoblasts in their lighter staining These cells are found occasionally in the blood of young infants but more frequently

singly or in groups These cytoplasmic inclusions are known as Russell bodies and consist of mucoprotein

Plasma cells are regarded by some as an independent series and by others as a form of lymphocyte [1] In either case the mature cells are derived from plasma blasts with intermediate forms constituting young plasma cells Plasma cells rarely appear normally in the peripheral blood but are present in the bone marrow lymph nodes spleen and other areas They are found in greatly increased numbers in the peripheral blood in patients with multiple myeloma plasma cell leukemia and to a lesser extent measles rubella chickenpox serum reactions and skin disorders The so called myeloma cell is a derivative of the early plasma cell and shows the main features of the plasmablasts

It seems well established that plasma cells are primarily responsible for the synthesis of the antibody components of gamma globulin perhaps to a greater extent than the lymphoid elements In patients with agammaglobulinemia there is a failure of plasma cell proliferation In biopsy specimens of lymph nodes in these patients no plasma cells or secondary follicle formation appears in response to injections of a bacterial vaccine and no antibody is formed[40] Good[31] failed to find plasma cells in the bone marrow of patients with agammaglobulinemia after infection and after the antigenic stimulation as occur in normal children

On the other hand hypergammaglobulinemia is most often associated with an increase of plasma cells in the lymph nodes spleen and bone marrow The concept that antibody and gamma globulin are produced by plasmacytes is given further support by the close correlation observed between the plasmacytic development in the bone marrow and increased gamma globulin production in patients suffering from acute rheumatic fever and in patients convalescent from streptoccal pharyngitis[3] Abnormal protein formation and marked hyperglobulinemia occur in patients with plasmacytic malignancies such as multiple myeloma and plasma cell leukemia

Turk s Cell (Turk Irritation Cell) Turk cells are the same size as plasma cells and are closely related to them They may be identical with the plasma cell an intermediate cell or represent an atypical lymphocyte The cytoplasm is nongranular and stains deeper blue than the plasma cell and the nucleus is eccentric but the chromatin is not cartwheel shaped These cells are increased in number in the peripheral blood of patients with severe anemias measles rubella agranulocytosis and chronic infections especially when associated with a marked leukocytosis

Rieder Cells Rieder cells are myeloblasts in which the nucleus is deeply indented resembling lobulation This conformation has been ascribed to the more rapid maturation of the nucleus than the cytoplasm

Auer Bodies Auer bodies or rods are small or elongated slender rods which stain red purple or azurophilic with Wright s stain and are peroxidase positive They are sometimes found singly or in larger numbers in the cytoplasm of myeloblasts myelocytes monoblasts monocytes and granular histiocytes (Ferrata cells) but not in the cytoplasm of lymphocytes or lymphoblasts Most commonly cells which contain Auer bodies are undifferentiated without granules in the cytoplasm and their presence in such cells is suggestive of leukemia

leukocytes in conjunction with abnormally formed or pyknotic nuclei The inclusions are usually larger and fewer in number than other types In one report three to six large irregular slate green masses were noted in the cytoplasm of the polynuclear neutrophils In some cells they were smaller and more numerous These inclusion bodies have been described in patients with malignant lymphoma and in conjunction with a hereditary pattern marked by semialbinism photophobia to direct light excessive sweating pale optic fundi hepatosplenomegaly and generalized lymphadenopathy [7 18]

Reilly Bodies In patients with gargoylism (Hurler s syndrome lipochondrodystrophy) a rare disease of early childhood characterized by dull coarse cretinoid facies dwarfism skeletal deformities clouding of the corneas and mental retardation fine and coarse blue colored granules have been described by Reilly in some 60 to 90 per cent of granulocytes and their precursors in the bone marrow and occasionally in the lymphocytes and monocytes [68 90] These granules are the same as those noted by Alder as a hereditary anomaly Patients with gargoylism have been described in whom the abnormal inclusions were confined to the lymphocytes the granulocytes being unaffected

CELL STAINS

Romanousky Stains Romanowsky stains of which Wright s stain is a modification consist of a combination of methylene blue with eosin Depending upon their acid and basic affinities the red and blue structures of cells are identified in contrasting colors The nuclei of white cells stain purplish blue with clear separation of basichromatin and oxychromatin neutrophil granules stain light pink or lavender and the granules of eosinophils and basophils stain red and deeply azurophilic respectively

Supravital Staining Supravital staining[10] permits the examination of cytoplasmic structures of blood cells in the living motile state In the combination employed the mitochondria are stained by Janus green and the specific granules and vacuoles of the cytoplasm by neutral red dye The nucleus and cytoplasm are left unstained but the nuclear outlines are readily discerned Mitochondria are most plentiful in the blast cells and decrease progressively as the cells mature being reduced in the late myelocytes and frequently absent in the mature polymorphonuclear leukocyte lymphocyte and monocyte

At times the blue green stain may serve in differentiating the blast cells by the shape and size of the mitochondria and their location In the myeloblast the mitochondria are numerous and extremely fine and are scattered diffusely or packed in a segment of the cytoplasm In the lymphoblast they are short thick oval or spherical and may be clustered around the nucleus or may be scattered diffusely In the monoblast they exist as fine slender rods scattered through the cell

Motility is absent in the blast forms but is present in varying degrees of ameboid motion in the mature cells of the three series most marked in the polymorphonuclear neutrophil In the monocyte the supravital stain is particularly diagnostic since the neutral red bodies are characteristically clustered as a rosette in the indentation of the nucleus Supravital staining does not replace fixed smear

in the bone marrow in patients with pure red cell (chronic aregenerative) anemia [77] lymphocytic leukemia and more particularly giant follicular lymphoblastoma in whom increased numbers have been observed in both the peripheral blood and bone marrow [71] The hematogone may be the same cell included in the classification of micromyeloblasts undifferentiated primordial cells or hemocytoblasts

Miscellaneous Inclusion Bodies Atypical granules or inclusion bodies have been described in all types of white blood cells especially in granulocytes

Alder's Anomaly Coarse dark azurophilic granules in the cytoplasm of the white cells especially the neutrophils occur as a rare hereditary anomaly without pathologic significance [41]

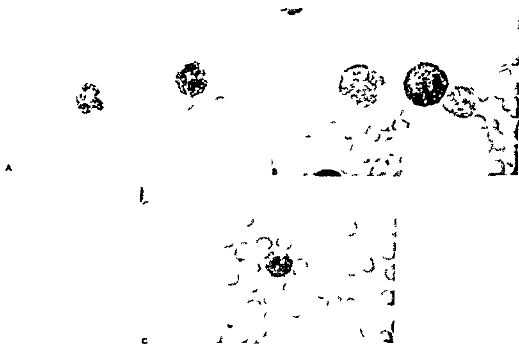


Fig 31 Blood and bone marrow smears from a boy 3 year old with gargoylism (Hurler's syndrome) Heavy granulation (Reilly bodies) present in polymorphonuclear leukocytes of the peripheral blood A myelocytes of the bone marrow B and lymphocytes C are characteristic of this disease These granules differ from toxic granulation and those found in the basophilic leukocytes Although coarse granules are common fine and medium granules may also be present (Courtesy Dr Ralph L Engle Jr New York N Y)

Dohle Bodies Round or oval shaped bodies of bluish cytoplasm either just visible or ranging in diameter to approximately 1 to 2 microns have been described in the cytoplasm of the polymorphonuclear neutrophil cells in patients with scarlet fever and other specific infections They appear during the first day or two after a burn and often disappear when the the skin is nearly completely covered [89] Amato bodies are probably the same as Dohle bodies (see p 337)

Chediak's Anomaly of Leukocytes These are Dohle like azurophilic granules ranging in size to 2 to 5 microns in diameter in the cytoplasm in all types of

18 Donohue W L and Bain H W Chediak Higashi Syndrome a Lethal Familial Disease With Anomalous Inclusions in the Leukocytes and Constitutional Stigmata Pediatrics 20 416 1957

19 Donohue W L Snelling C E Jackson S H Keith J D Chute A L Laski B and Silverthorne N Pituitary Adrenocorticotropic Hormone (ACTH) Therapy in Eosinophilic Leukemia J A M A 143 154 1950

19a Dorr A D and Moloney W C Acquired Pseudo Pelger Anomaly of Granulocytic Leukocytes New England J Med 261 742 1959

20 Dougherty T F and White A Functional Alterations in Lymphoid Tissue Induced by Adrenal Cortical Secretion Am J Anat 77 81 1945

21 White A Influence of Endocrine Secretions on the Structure and Function of Lymphoid Tissue Harvey Lect p 43 1947 1948

22 Efrati P and Jonas W Chediak's Anomaly of Leukocytes in Malignant Lymphoma Associated With Leukemic Manifestations Case Report With Necropsy Blood 13 1063 1958

23 Epstein W L and Kligman A W Pathogenesis of Eosinophilic Pneumonitis (Loeffler's Syndrome) J A M A 162 95 1956

24 Esselier A F Jeanneret R L and Morandi L The Mechanism of Glucocorticoid Eosinopenia Contribution to the Physiology of Eosinophile Granulocytes Blood 9 531 1954

25 Evans F S and Nesbit R R Eosinophilic Leukemia Report of a Case With Autopsy Confirmation Review of Literature Blood 4 603 1949

26 Farquhar J W The Evaluation of the Eosinopenic Response to Corticotrophin and Cortisone in the Newborn Infant Arch Dis Childhood 30 133 1955

27 Fessas P Wintrobe M M Thompson R B and Cartwright G E Treatment of Leukemia With Cortisone and Corticotrophin A M A Arch Int Med 94 384 1954

28 Finch S C Ross J F and Ebaugh F G Immunologic Mechanisms of Leucocyte Abnormalities J Lab & Clin Med 42:555 1953

29 First Report of the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood Forming Organs Am J Clin Path 18 443 1948

30 Good R A Agammaglobulinemia Bull Univ Minnesota Hosp 26 1 1954

31 Good R A Studies on Agammaglobulinemia Failure of Plasma Cell Formation in the Bone Marrow and Lymph Nodes of Patients With Agammaglobulinemia J Lab & Clin Med 46 167 1955

32 Good R A and Campbell B Correlation of the Plasmacytosis of the Bone Marrow With the Modifications of the Gamma Globulin of the Serum in Rheumatic Fever Am J Med 9 330 1950

33 Gordin R Toxic Granulation in Leukocytes Development and Relation to Cloudy Swelling Acta med scandina (suppl 270) 143 1 1952

34 Hargraves M M Richmond H and Morton R Presentation of Two Bone Marrow Elements Tart Cell and L E Cell Proc Staff Meet Mayo Clin 23 25 1948

35 Harris S and Harris T N Studies on the Transfer of Lymph Node Cells Effects of *Variation in the Interval Between the Injection of Antigen Into the Donor and Collection* of Its Lymph Node Cells J Exper Med 100 269 1954

36 Haserick J R and Sundberg R D Bone Marrow as Diagnostic Aid in Acute Disseminated Lupus Erythematosus Report on Hargraves L E Cell J Invest Dermat 11 209 1948

37 Heiner D C and Kevy S V Visceral Larva Migrans New England J Med 254 629 1956

37a Holman H Systemic Lupus Erythematosus a Review of Certain Recent Developments in the Study of This Disease J Pediat 56 109 1960

38 Holman H R and Kunkel H G Affinity Between the Lupus Erythematosus Serum Factor and Cell Nuclei and Nucleoprotein Science 126 162 1957

39 The Lupus Erythematosus Test Queries and Minor Notes J A M A 151:1460 1953

but serves as an adjunct in the differentiation of cells and as an aid in visualizing their physiologic activity

Peroxidase Stain The presence of an oxidating ferment in the cytoplasm of myeloid cells provides an added means of differentiating these from other cells In the commonly used techniques (such as the Goodpasture and the copper peroxidase method of Sato and Sekiya) this ferment causes the oxidation and precipitation of benzidine by hydrogen peroxide Cells of the granulocytic series including promyelocytes give a strong peroxidase reaction as contrasted with lymphocytes plasma cells and red cells which are peroxidase negative Monocytes show fewer and less well defined peroxidase positive granules than do granulocytes Myeloblasts give a negative reaction hence this reaction is of no value in separating these cells from lymphoblasts

REFERENCES

1 Ambrus C N Ambrus J C Johnson C C Packman E W Chernick W S Back N and Harrisson J W E Role of the Lungs in Regulation of the White Blood Cell Level Am J Physiol 178 33 1954

2 Bass M H Extreme Eosinophilia and Leukocytosis Am J Dis Child 62 68 1941

3 Batson R Shapiro J Christie A and Riley H D Jr Acute Non lipid Disseminated Reticuloendotheliosis A M A Am J Dis Child 90 323 1955

4 Bierman H R Kelly K H and Cordes F L The Sequestration and Visceral Circulation of Leukocytes in Man Ann New York Acad Sc 59 850 1955

5 Bierman H R Kelly K H Cordes F L Byron R L Jr Polhemus J A and Rappoport S The Release of Leukocytes and Platelets From the Pulmonary Circulation by Epinephrine Blood 7 683 1952

6 Bonnin J A and Schwartz L The Combined Study of Agglutination Hemolysis and Erythrophagocytosis With Special Reference to Acquired Hemolytic Anemia Blood 9 773 1954

7 Chediak M M Nouvelle anomalie leucocytaire de caractere constitutionnel et familial Rev hemat 7 362 1952

8 Crofton J W Livingstone J L Oswald N C and Roberts A T M Pulmonary Eosinophilia Thorax 7 1 1952

9 Cunningham R S Sabin F R Sugiyama S and Kindwall J A Role of the Monocyte in Tuberculosis Bull Johns Hopkins Hosp 37 231 1925

10 Cunningham R S and Tompkins F H The Supravital Staining of Normal Human Blood Cells Folia haemat 42 257 1950

11 Dameshek W and Reeves W H Exacerbation of Lupus Erythematosus Following Splenectomy in Idiopathic Thrombocytopenic Purpura and Autoimmune Hemolytic Anemia Am J Med 21 560 1956

12 Dacie J M Dacie J V and McSorley J C A Pelger Like Leucocytes in Chronic Myeloid Leukemia Acta haemat 12 117 1954

13 Dausset J Nenna A and Brecy H Leukoagglutinins Leukoagglutinins in Chronic Idiopathic and Symptomatic Pancytopenia and in Paroxysmal Nocturnal Hemoglobinuria Blood 9 696 1954

14 Davidson W M and Smith D R A Morphological Sex Difference in the Polymorphonuclear Neutrophil Leucocytes Brit M J 2 6 1954

15 Doan C A Neutropenic State Its Significance and Therapeutic Rationale J A M A 99 194 1932

16 Doan C A The White Blood Cells in Health and Disease Bull New York Acad Med 30 415 1954

17 Doan C A and Wiseman B K The Monocyte Monocytosis and Monocytic Leukoses Ann Int Med 8 383 1934

63 Osgood, E. E. Seaman, A. J., Tivey H., and Regas, D A. Duration of Life and of Different Stages of Maturation of Normal and Leukemic Leukocytes Rev hemat 9 543, 1954
64 Osgood, E. E Tivey H., Davison, K. B., Seaman, A. J., and Li, J G. Relative Rates of Formation of New Leukocytes in Patients With Acute and Chronic Leukemias Measured by *Uptake of Radioactive Phosphorus in Isolated Desoxyribosenucleic Acid*, Cancer 5 331 1952
65 Ottesen, J On the Age of Human White Cells in Peripheral Blood, Acta physiol. scandinav 32 75 1954
66 Padawer J Studies on Mammalian Mast Cells Tr New York Acad. Sc 19 690, 1957
67 Pearse A. G E. The Nature of Russell Bodies and Kurloff Bodies J Clin. Path 2 81 1949
68 Reilly W A. The Granules in the Leukocytes in Gargoylism Am J Dis Child. 62 489 1941
69 Riley J F Heparin, Histamine and Mast Cells Blood 9 1123 1954
70 Rosenfeld, S Swiller A I and Morrison M Simple Method of Demonstrating the L.E. Cell by Finger Puncture J.A.M.A. 155 568 1954
71 Rosenthal K. Dreskin O H., Vural I. L. and Zak, F G The Significance of *Hematogones in Blood, Bone Marrow and Lymph Node Aspiration in Giant Follicular* Lymphoblastoma, Acta haemat 8 368 1952.
72. Ross S W., and Wells B B Systemic Lupus Erythematosus a Review of Literature Am J Clin. Path 23 139 1953
73 Rosse W F and Gurney C W The Pelger Huet Anomaly in Three Families and Its Use in Determining the Disappearance of Transfused Neutrophils From the Peripheral Blood, Blood 14 170 1959
74 Rowley D A. and Benditt, E P 5-Hydroxytryptamine and Histamine as Mediators of *the Vascular Injury Produced by Agents Which Damage Mast Cells in Rats*, J Exper Med. 103 399 1956
75 Silver H K. Henderson P and Contopoulos A. Extreme Eosinophilia Increased Blood Heterophile-Agglutination Titer and Hyperglobulinemia A.M.A Am J Dis Child. 83 649 1952.
76 Smith, C. H The Leukocytic Reaction in Tuberculosis of Infancy and Childhood Am J M Sc 182 221 1931
77 Smith C H The Anemias of Early Infancy J Pediat 16 375 1940
78 Smith H D and Beaver P C Visceral Larva Migrans Due to Infection With Dog and Cat Ascarids Pediat Clin North America 2 163 1955
79 Speirs R S Physiological Approaches to an Understanding of the Function of Eosinophils and Basophils Ann New York Acad Sc 59 706 1955
80 Stedman E and Stedman E The Cytological Interpretation of the Feulgen Reaction Biochem J 47 508 1950
81 Steinberg B and Martin R A Plasma Factor Increasing Circulatory Leukocytes Am J Physiol 161 14 1950
82. *Teneczar F J and Streitmatter D E Sex Difference in Neutrophils* Am J Clin Path 26 384 1956
83 Thorn G W Forsham P H Prunty F T G and Hills A G A Test for Adrenal Cortical Insufficiency the Response to Pituitary Adrenocorticotropic Hormone J A M.A 137 1005 1948
84. Ullrich O and Wiedemann H R Promyelozyten Erythrophagie Acta haemat 11 134 1954
85 van der Sar A. The Pelger Huet s Familial Nuclear Anomaly of the Leucocytes Am J Clin Path 14 544 1944
86 Walsh J R and Zimmerman H J Demonstration of L E Phenomenon in Patients With Penicillin Hypersensitivity Blood 8 651 1953
87 Waters W J and Lacson P S Mast Cell Leukemia Presenting as Urticaria Pigmentosa Report of a Case Pediatrics 19 1033 1957

40 Janeway C A and Citlin D The Gamma Globulins In Levine S Z (editor) Advances in Pediatrics vol 9 Chicago 1957 Year Book Publishers Inc p 65
41 Jordans G H W Hereditary Granulation Anomaly of the Leucocytes (Alder) Acta med scandinav 129 348 1947
42 Lapin J and Horonick A Ameboid Motility of Human Leukocytes Blood 11 225 1956
43 Lawrence J S Physiology and Functions of the White Blood Cells J A M A 157 1212 1955
44 Lehndorff H Transitorische Granulocytopenie beim Neugeborenen Helvet paediat acta 6 173 1951
45 Lichtenstein L Histiocytosis Integration of Eosinophilic Granuloma of Bone Letterer Siwe Disease and Schuller Christian Disease as Related Manifestations of a Single Nosologic Entity A M A Arch Path 56 84 1953
45a McCall M S Sutherland D A Eisentraut A and Lanz H The Tagging of Leukemic Leukocytes With Radioactive Chromium and Measurement of the In Vivo Cell Survival J Lab & Clin Med 45 717 1955
46 Mallory T B Pathology Diseases of Bone New England J Med 227 955 1942
47 Mathis H B A Simple Office Procedure for Demonstrating Lupus Erythematosus Cells in Peripheral Blood Blood 6 470 1951
48 Matoth Y Phagocytic and Ameboid Activities of the Leukocytes in the Newborn Infant Pediatrics 9 748 1952
49 Menken V The Determination of the Level of Leukocytes in the Blood Stream With Inflammation a Thermostable Component Concerned in the Mechanism of Leukocytosis Blood 4 1323 1949
50 Michael S R Vural I L Bassen F A and Schaefer L Hematologic Aspects of Disseminated (Systemic) Lupus Erythematosus Blood 6 1059 1951
51 Michels N A The Plasma Cell Arch Path 11 775 1931
52 Miescher P Nucleophagocytose et phenomene L E Schweiz med Wchnschr 83 1042 1953
53 Miller G L Clinical Significance of the Blood in Tuberculosis New York 1943 Commonwealth Fund
54 Miller J L Brodey M and Hill J H Studies on Significance of Biologic False Positive Reactions J A M A 164 1461 1957
55 Mitchell B Basophilic Leukocytes in Children in Health and Disease Arch Dis Childhood 33 193 1958
55a Mittwoch U Abnormal Lymphocytes in Gargoylism Brit J Haemat 5 365 1959
56 Moeschlin S and Wagner K Agranulocytosis Due to Occurrence of Leukocyte agglutinins (Pyramidon and Cold Agglutinins) Acta haemat 8 29 1952
57 Moeschlin S Meyer H Israels L B and Tarr Gloor E Experimental Agranulocytosis Its Production Through Leukocyte Agglutination by Anti leukocytic Serum Acta haemat 11 73 1954
58 Moore J E and Lutz W B The Natural History of Systemic Lupus Erythematosus an Approach to Its Study Through Chronic Biologic False Positive Reactors J Chron Dis 1 297 1955
59 Moore J E Shulman L E and Scott J T The Natural History of Systemic Lupus Erythematosus an Approach to Its Study Through Chronic Biologic False Positive Reactors J Chron Dis 5 282 1957
60 Moore T D A Simple Technique for the Diagnosis of Nonlipid Histiocytosis Pediatrics 19 438 1957
61 Orchard N P Letterer Siwe's Syndrome Report of a Case With Unusual Peripheral Blood Changes Arch Dis Childhood 25 151 1950
62 Osgood E E Li J G Tivey H Duerst M L and Seaman A J Growth of Human Leukemic Leukocytes in Vitro and in Vivo as Measured by Uptake of P³² in DNA Science 114 95 1951

CHAPTER 19

Leukopenia and Leukopenic Syndromes

Leukopenia denotes the condition in which the number of circulating white blood cells is below 4 000 per cubic millimeter without reference to the type of leukocyte involved Except when the leukocyte count is exceedingly low and all types of white blood cells are reduced leukopenia is usually due to a reduction in the neutrophils Infrequently the lymphocytes are markedly reduced, resulting in alymphocytosis[3] On the other hand, in some instances there may be neutropenia without leukopenia This is seen occasionally in serial white blood counts of patients with many conditions of protracted leukopenia, although the granulocytes are consistently reduced In such an event the bulk of cells are made up of lymphocytes occasionally with monocytosis

Pathogenesis Leukopenia is brought about by a variety of mechanisms[1] These include diminished manufacture of white blood cells as a result of simple inhibition (for example overwhelming infection septicemia)[2] maturation arrest (for instance agranulocytosis) aplasia of the marrow (for example aplastic anemia) damage or destruction of leukocyte forming tissue (such as that caused by chemical and physical agents) destruction or inhibitory effect of the spleen on granulocytes (that is splenic neutropenia) or direct action on the peripheral blood by leukocyte agglutinins A redistribution of the white cells conceivably also may cause peripheral leukopenia by withdrawing granulocytes from vascular channels to localized areas of pus formation Replacement of the bone marrow by leukemic or neoplastic cells or by cells of entities such as Gaucher s or Hodgkin s disease results in leukopenia in some phase of the respective diseases The course is unknown but has been attributed to crowding of myeloid tissue or a disturbance of local metabolism by the rapid multiplication of invading cells In some cases the concept of hypersplenism is involved

Leukocyte agglutinating factors responsible for the production of agranulocytosis and leukopenia have been demonstrated in the serum of patients The formation of antibodies capable of agglutinating white blood cells in the blood stream has led to the concept of immunoagranulocytosis or immunoleukopenia This phenomenon is comparable to autoimmune mechanisms involved in the produc

88 Watkins C H and Hall B E Monocytic Leukemia of the Naegeli and Schilling Types Am J Clin Path 10 387 1940
89 Weiner W and Topley E Dohle Bodies in the Leucocytes of Patients With Burns J Clin Path 8 324 1955
90 Wiedemann H R Beitrage zur Pfaundler Hurlerschen Krankheit Ztschr Kinderh 70 81 1951
91 Wolman B The Function of the Adrenal Glands in the Newborn Arch Dis Childhood 27 283 1952
92 Zimmerman H J Walsh J R and Heller P Production of Nucleophagocytosis by Rabbit and Antileukocytic Serum Blood 8 651 1953
93 Zinkham W H and Conley D D Some Factors Influencing the Formation of L E Cells a Method for Enhancing L L Cell Production Bull Johns Hopkins Hosp 98 102 1956
94 Zinkham W H and Diamond L K In Vitro Erythrophagocytosis in Acquired Hemolytic Anemia Blood 7 592 1952
95 Zuelzer W W and Apt L Disseminated Visceral Lesions Associated With Extreme Eosinophilia Am J Dis Child 78 153 1949

Infections A persistent leukopenia may indicate a serious prognosis in the presence of certain infections in which leukocytosis ordinarily is expected.[29] Its presence is therefore of diagnostic value in patients with bacterial infections such as typhoid paratyphoid, and undulant fever with certain virus diseases such as influenza, measles rubella, and primary atypical pneumonia, with protozoal infections such as malaria and with the early stage of rickettsialpox.

Leukopenia is noted in about one third of patients with disseminated lupus erythematosus on initial examination and develops subsequently in about one half sometime in the course of the disease[1] A moderate increase in nonsegmented neutrophils is seen in most patients

Chemicals Drugs and Irradiation Chemicals and drugs such as anticonvulsants antimicrobial agents (that is chloramphenicol) antithyroid drugs (that is thiouracil and its derivatives) tranquilizers[2] antihistamines (that is Pyribenzamine) and sulfonamides are occasionally associated with leukopenia and neutropenia. Exposure to physical agents such as irradiation and to chemicals such as antimetabolites nitrogen mustards Myleran and 6-mercaptopurine which are employed in the therapy of leukemia and neoplastic disease can be expected to produce leukopenia and a hypoplastic bone marrow when given in sufficient dosage and over a variable length of time Depending upon individual susceptibility and duration of exposure benzol results in leukopenia, agranulocytosis and a complete picture of aplastic anemia. In hypersensitive persons aminopyrine and related antipyrine may cause serious and even fatal leukopenia and agranulocytosis

Not only do these chemicals exert a destructive effect upon myelopoietic cells in the bone marrow but also as stated previously with regard to drug sensitive persons agglutinins can account for peripheral destruction of leukocytes According to this concept bone marrow depletion and exhaustion result secondarily from the accelerated destruction of granulocytes in the peripheral blood.

The lymphocyte is the most radiosensitive of all white blood cells and the most important change in the white blood cell picture after exposure to radiation is a reduction in the total number of these cells This is principally due to a decrease in the number of granulocytes which numerically represent the majority of blood cells in the average human being.[13]

Miscellaneous The miscellaneous causes of leukopenia include a wide variety of conditions

AGRANULOCYTOSIS The incidence of fatal agranulocytosis is greater in adults than in children. Secondary neutropenia may occur following the administration of drugs or following infections which damage the bone marrow Although the disease occurs primarily in adults greater heed should be given this condition in childhood because of the increasing use of drugs and antimicrobial and antibiotic agents

Agranulocytosis is an acute febrile disease with a high mortality rate characterized by leukopenia, pronounced reduction in neutrophils and slight if any changes in the red blood cells or platelets An important clinical feature is the necrotic lesions in the throat and elsewhere Most cases of agranulocytosis are attributed to the effect of one of a number of drugs in a susceptible person

tion of certain types of idiopathic hemolytic anemia and thrombopenic purpura. Antileukocyte activity has been demonstrated in the serums from patients with a wide variety of hematologic diseases with leukopenia, especially in those with a hyperplastic marrow.[30] The majority of these agglutinins are blood group specific, and a small percentage are nonspecific (globulins).[18] This agglutinating mechanism has been applied to the leukopenic effect of drugs and chemicals,[19] to idiopathic immunoneutropenia,[1] and to the development of the L.E. phenomenon in disseminated lupus erythematosus.[6] Transitory granulocytopenia of the newborn infant (neonatal agranulocytosis) has also been explained on the basis of immunoleukopenia.[14, 6] Leukoagglutinins occur more frequently among persons who have had transfusions, especially repeated transfusions—hence the possibility that they represent antibodies to antigens of leukocytes.[3]

Bone Marrow. Although the bone marrow reflects the state of granulopoiesis, the bone marrow findings cannot always be correlated with those in the peripheral blood. The combined examination is necessary, however, for interpretation and management of a leukopenic condition.

The bone marrow ranges from the normal to a maturation arrest at the promyelocytic level. The variability depends upon the stage, duration, and severity of the disease when the bone marrow is examined. Thus the peripheral leukopenia of severe infection may be unassociated with alteration of the bone marrow, and the reason for the leukopenia is unexplained. On the other hand, in patients with *chronic neutropenia the bone marrow may reveal few myelocytes,* promyelocytes, and infrequent myeloblasts with an increase in reticular cells, lymphoid cells, and plasma cells. In other cases a true maturation arrest occurs with striking hyperplasia of early myelocytes with a conspicuous absence of mature granulocytes.

Causes. The causes of leukopenia may be summarized as follows:

1. Infections
   - A. Bacterial
   - B. Viral
   - C. Miscellaneous
     - (1) Protozoal
     - (2) Rickettsial
   - D. Overwhelming
2. Chemicals, drugs, and physical agents
   - A. Irradiation
   - B. Therapeutic agents in systemic and blood disease
3. Miscellaneous
   - A. With blood disorders
     - (1) Agranulocytosis
     - (2) Aplastic anemia and leukemia
     - (3) Hypoplastic anemia and leukopenia
     - (4) Periodic (cyclic) neutropenia
     - (5) Chronic hypoplastic neutropenia
     - (6) Chronic benign granulocytopenia in childhood
     - (7) Infantile genetic agranulocytosis
     - (8) Neonatal agranulocytosis
   - B. Involving the spleen
     - (1) Primary and secondary splenic neutropenia
   - C. Nutritional deficiencies

PERIODIC (CYCLIC) NEUTROPENIA Periodic neutropenia is a rare condition characterized by extreme leukopenia due to the disappearance of neutrophilic granulocytes from the circulating blood at approximately three week intervals and occurring at any age period but frequently beginning in infancy and childhood. The agranulocytic periods last ten days and are associated with the appearance of ulcers in the oral mucous membranes fever and sore throat Splenomegaly adenopathy arthralgia, abdominal pain, ischiorectal infections lymphadenitis, and conjunctivitis have also been reported[2–4] As in patients with other kinds of leukopenia, when the white count is lowest mouth ulcerations gingivitis and often attacks of furunculosis are prone to appear

In general the fluctuations of the circulating neutrophils are a reflection of the cyclic changes in the bone marrow The granulocytes and their precursors may disappear from the bone marrow before the onset of neutropenia and reappear just prior to the return of neutrophils in the peripheral blood.[2] Often the bone marrow is depleted of mature forms during the agranulocytic stage but an abundance of early granulocytic forms constituting a "maturation arrest" is found

Recurrent neutropenia has been described [1] in a patient with onset of disease in infancy marked by furunculosis, aphthous stomatitis and fever who was followed until death as a young adult. The three week cycle of neutropenia persisted with the leukocytes during these episodes numbering 2,000 to 4 000 per cubic millimeter and the granulocytes 6 to 10 per cent.

The pathogenesis of these syndromes the reason for their periodicity and the tendency for ulceration of the buccal mucosa have not been explained No definite relationship to hormonal imbalance or the menstrual cycle has been established It is not yet clear whether antileukocytic antibodies play a role in causation

CHRONIC HYPOPLASTIC NEUTROPENIA In chronic hypoplastic neutropenia the course is extremely chronic and marked by repeated infections involving the skin and oral cavity and frequently by symptom free intervals Splenomegaly of slight to moderate degree is present in all patients The marked hypoplasia of granulocytic precursors of the bone marrow and the failure to respond to splenectomy differentiates this condition from primary splenic neutropenia in which granulocytic hyperplasia is present in the marrow and benefit is obtained by splenectomy

CHRONIC BENIGN GRANULOCYTOPENIA IN CHILDHOOD Chronic benign granulocytopenia with leukopenia has been reported frequently The bone marrow in these patients is usually normal but in some there is a disturbance in maturation of myelocytes to the segmented forms Spontaneous remissions with eventual recovery may occur in a period of months to years [31] In some children there is a lowered resistance to infection whereas in others neutropenia with leukopenia which persists for years without causing ill health but with a transient rise of granulocytes with severe infection is discovered as a chance finding In one such child the granulocytes numbered below 10 per cent, with white blood counts ranging from those observed in leukopenia to normal counts This persisted from the ages of 2 to 7 years with a complete and lasting remission following tonsillectomy Rarely chronic neutropenia occurs in several siblings of a family

The bone marrow usually shows a reduction in the total count of nucleated cells and a depression of the myeloid elements leaving the red cell precursors and megakaryocytes relatively undisturbed. The pathologic changes in the bone marrow are extremely variable and depend upon the stage of the disease when bone marrow is aspirated. Following injury there is a gradual disappearance of the myeloblasts, promyelocytes, myelocytes and metamyelocytes and eventually of the more mature neutrophilic cells. These elements are replaced in the bone marrow by plasma cells, reticuloendothelial elements and lymphocytes. Occasionally in this phase a proliferation of myeloblasts occurs. During recovery and in the patient with the mild form that has not progressed the less mature myeloid forms reappear and are followed by the segmented neutrophilic cells.

Repeated bone marrow studies are required, however, to guard against a sudden shift to an immature level. In patients with mild injury both promyelocytes and myelocytes are present and a marked reduction in the more mature elements and an increase in stem cells and lymphocytes require immediate withdrawal of the offending drug. The prognosis, therefore, is favorable if normal numbers of granulocytes are observed in the marrow and is unsatisfactory if only lymphocytes and plasma cells are present.

Since the bone marrow returns to normal before mature granulocytes are liberated in the peripheral blood, prognosis depends upon the cellular composition of the marrow. Except for antimicrobial agents and antibiotics, the effectiveness of the drugs usually prescribed for treatment of agranulocytosis must be correlated with the myelopoietic content of the marrow.

When penicillin or other antibiotics administered to combat infection are found to cause agranulocytosis, recovery from the agranulocytosis following their withdrawal probably is spontaneous and basically depends upon the ability of intrinsic factors supplied by the patient to stimulate granulopoiesis.

LEUKOPENIA IN APLASTIC ANEMIA AND LEUKEMIA. In children a persistent neutropenic leukopenia with or without purpura frequently signifies the presence of aplastic anemia or leukemia. One of the commonest features of childhood leukemia is the occurrence of leukopenia with predominance of lymphocytes and a moderate anemia during the course of an unexplained low grade fever associated with bouts of markedly elevated temperatures. Examination of the peripheral blood normally reveals a sparsity of platelets and occasionally immature white blood cells, whereas bone marrow examination usually demonstrates heavy infiltration with leukemic cells.

HYPOPLASTIC ANEMIA AND LEUKOPENIA. Leukopenia may occur in patients with mild hypoplastic anemia. In one instance fatal idiopathic aplastic anemia occurred in first cousins and a mild anemia and protracted leukopenia which extended from childhood into adult life was observed in a sister of one of them. In addition to oral ulceration, susceptibility to furunculosis and skin infiltration is common with intervals of good health between episodes despite the persistent leukopenia. The bone marrow in these patients shows a greater proportion of myeloid cells of all grades of maturity. A similar bone marrow picture was observed in a child with persistent leukopenia and neutropenia in whom febrile episodes occurred when the leukopenia was most marked.

PERIODIC (CYCLIC) NEUTROPENIA Periodic neutropenia is a rare condition characterized by extreme leukopenia due to the disappearance of neutrophilic granulocytes from the circulating blood at approximately three week intervals and occurring at any age period but frequently beginning in infancy and childhood The agranulocytic periods last ten days and are associated with the appearance of ulcers in the oral mucous membranes fever and sore throat Splenomegaly adenopathy arthralgia abdominal pain ischiorectal infections lymphadenitis and conjunctivitis have also been reported [4] As in patients with other kinds of leukopenia when the white count is lowest mouth ulcerations gingivitis and often attacks of furunculosis are prone to appear

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ranging in severity from the mild to fatal case Continuous treatment with antibiotics has been recommended as a means of rendering the child with chronic neutropenia free from infections [16]

A form of this disease has been reported in adults whose course is marked by attacks of severe infection from relatively trivial causes Cutaneous sepsis and inflammation of the gums lips mouth and throat are common with free intervals of comparative good health [1] The bone marrow varies from hypoplasia to hyperplasia

INFANTILE GENETIC AGRANULOCYTOSIS A variant of chronic benign granulocytopenia in childhood is infantile genetic agranulocytosis in which a severe depletion of granulocytes frequently with leukopenia is transmitted on the basis of simple recessive inheritance [11]

NEONATAL AGRANULOCYTOSIS Lubhy and Slobody[14] observed transient agranulocytosis in successive siblings in the neonatal period Such a circumstance was explained by transplacental isoimmunization of the mother to a leukocyte factor of her infant in a manner analogous to Rh isoimmunization causing hemolytic disease of the newborn infant [74] In these patients agranulocytosis persisted for three to four weeks and in one of the infants it was accompanied by pulmonary infection The predominance of neutrophilic myelocytes in the bone marrow may represent either a maturation arrest or a depletion of mature cells because of their increased agglutination and destruction in the peripheral blood

Infants born to mothers with chronic neutropenia have revealed a transitory neutropenia which presumably was caused by the transplacental passage of a neutropenic factor [8] In one case the mother's serum contained a demonstrable leukoagglutinin which could be transferred by a transfusion of plasma to a normal donor with a resulting severe neutropenia The neonatal neutropenia persisted for three weeks Hyperplasia of the granulocytic series with a maturation arrest at the myelocytic stage was present in the bone marrow of the affected infants

Multiple cases of neonatal neutropenia occurring in one family were traced to a potent leukoagglutinin found in the maternal serum which agglutinated the leukocytes obtained from the father and three available children [11a] On the other hand leukocyte agglutinins were absent in a patient with severe granulocytopenia associated with infection from 1 week of age which terminated fatally at 13½ months of age [8] The serum electrophoretic pattern was normal The bone marrow revealed an arrest at the myelocytic stage

SPLENIC NEUTROPENIA Primary splenic neutropenia[3] is characterized by profound leukopenia and neutropenia fever ulcerations of the mucous membranes susceptibility to infection splenomegaly and myeloid hyperplasia of the bone marrow in which there may be an associated depression of the red cells and platelets This has been regarded as a form of hypersplenism in which the leukocytes are involved in splenic hyperactivity Relief of all symptoms results from splenectomy In patients with secondary splenic neutropenia leukopenia occurs with disorders involving the spleen such as Banti's disease (portal hypertension) Gaucher's disease lymphosarcoma and Hodgkin's disease It is not yet certain whether the leukopenia in the primary or secondary syndromes results from

hypersequestration and hyperdestruction of granulocytes by and within the spleen or from inhibition by the spleen through a hormonal effect or by both mechanisms

ALYMPHOCYTOSIS Alymphocytosis is a rare fatal disease occurring in infants and young children[3] which is characterized by fever hepatosplenomegaly and leukopenia with an absolute reduction of all types of leukocytes especially the lymphocytes Pancytopenia occurs terminally Postmortem examination reveals a widespread and generalized atrophy and necrosis of lymphoid tissue Infection with *Candida albicans* (moniliasis) was a common factor in patients reported on From their pathologic findings Janeway and Gitlin[9] suspect the presence of agammaglobulinemia

NUTRITIONAL DEFICIENCIES In experimental animals nutritional deficiencies induce leukopenia and granulocytopenia Leukopenia granulocytopenia, and occasionally anemia develop in rats fed a purified diet deficient in riboflavin[4] Folic acid corrects leukopenia and granulocytopenia can be corrected by the administration of protein[10] In patients in whom white blood cell regeneration was promoted by dietary protein granulocytes were found to react to a greater degree than lymphocytes and monocytes[8]

Treatment In deciding on treatment it must be remembered that many diseases such as leukemia aplastic anemia disseminated lupus erythematosus and lymphosarcoma may be preceded by a period of prolonged and undiagnosed leukopenia One child with neutropenia and repeated respiratory infections who was followed from 3 to 6 years of age eventually succumbed with a pathologic diagnosis of lymphosarcoma In this patient splenectomy was unsuccessful in altering the blood picture

The first steps in the treatment of neutropenia should aim toward the elimination of suspected drugs or chemicals and removal of the patient to an environment free from possible exposure to offending agents In the event of recurrent infections antibiotics should be prescribed to offset the effect of granulocytopenia with the hope that myeloid tissue will regenerate spontaneously Mouth ulcerations require urgent local treatment

Etiologic agents are usually difficult to ascertain and the problem of therapy is one of stimulating granulocytic proliferation Unfortunately such specific agents are not yet available and treatment on the whole is unsatisfactory Definitive treatment consists of prophylactic and therapeutic administration of antibiotics and the adrenocortical hormones and splenectomy None of these measures significantly alters the blood picture of patients with the cyclic or other neutropenias although ACTH the steroid hormones and splenectomy produce some degree of symptomatic relief especially with respect to fever and oral ulcers Pentnucleotide liver extract, vitamins and bone marrow extracts and leukocytic products have been ineffective in reversing leukopenia and granulocytopenia

Permanent and spontaneous remissions have been observed after prolonged periods of leukopenia especially when recurrent infections have been mild No clear-cut distinction can always be made between clinical syndromes that have in common leukopenia and granulocytopenia Attempts are usually made to

explain the leukopenia on the basis of splenic hyperactivity Only in patients with primary splenic neutropenia when all criteria are fulfilled does removal of the spleen lead to complete recovery[3] In other patients in whom the bone marrow shows a myelocytic hyperplasia without splenic enlargement removal of the spleen only rarely results in improvement without altering the basic condition [6]

REFERENCES

1 Adams E B and Witts L J Chronic Agranulocytosis Quart J Med 18 173 1949
2 Bigler J A and Brennenmann J Sepsis With Leukopenia (Agranulocytosis) in Children Report of Ten Cases Am J Dis Child 40 515 1939
3 Donohue W L Alymphocytosis Pediatrics 11 129 1953
4 Endicott K M Kornberg A and Ott M Hemopoiesis in Riboflavin Deficient Rats Blood 2 164 1947
5 Fanconi G Klinische Demonstrationen Ann paediat 157 308 1941
6 Finch S S Ross J F and Ebaugh F G Immunologic Mechanisms of Leukocyte Abnormalities J Lab & Clin Med 42 555 1953
7 Gasser C Die Pathogenese der essentiellen chronischen Granulocytopenie im Kindesalter auf Grund der Knochenmarksbefunde Helvet paediat acta 7 426 1952
8 Guggenheim K and Buechler S The Effect of Quantitative and Qualitative Protein Deficiency on Blood Regeneration White Blood Cells Blood 4 958 1954
8a Hedenberg F Infantile Agranulocytosis of Probable Congenital Origin Acta paediat 48 77 1959
9 Janeway C A and Gitlin D The Gamma Globulins In Levine S Z (editor) Advances in Pediatrics vol 9 Chicago 1957 Year Book Publishers Inc p 65
10 Kornberg A J Daft F S and Sebrell W H Granulocytopenia and Anemia in Rats Fed Diets of Low Casein Content Science 103 646 1946
11 Kostmann R Infantile Genetic Agranulocytosis a New Recessive Lethal Disease in Man Acta paediat (suppl 105) 45 1 1956
11a Lalezari P Nussbaum M Gelman S and Spaet T H Neonatal Neutropenia Due to Maternal Isoimmunization Blood 15 236 1960
12 Lawrence J S Leukopenia a Discussion of Its Various Modes of Production J A M A 116 478 1941
13 Lawrence J S Adams W S and Valentine W N White Blood Cell Changes in Clinical Disorders J A M A 150 454 1952
14 Lubby A L and Slobody L B Transient Neonatal Agranulocytosis in Two Siblings Transplacental Isoimmunization to a Leukocyte Factor? A M A Am J Dis Child 92 496 1956
15 Martensson J and Vikbladh I Idiopathic Immunoneutropenia Report of a Case With Leukocyte Agglutinating Factor in the Serum Blood 9 632 1954
16 McLean M M Chronic Neutropenia Arch Dis Childhood 32 431 1957
17 Michael S R Lutfi Vural I Bassen F A and Schaefer L The Hematologic Aspects of Disseminated (Systemic) Lupus Erythematosus Blood 6 1059 1951
18 Moeschlin S and Schmid E Investigation of Leukocyte Agglutination in Serum of Compatible and Incompatible Blood Group Acta haemat 11 241 1954
19 Moeschlin S and Wagner K Agranulocytosis Due to the Occurrence of Leukocyte Agglutinins Acta haemat 8 29 1952
20 Moeschlin S Meyer H Israels L C and Tarr Cloor E Experimental Agranulocytosis Its Production Through Leukocyte Agglutination by Antileukocytic Serum Acta haemat 11 73 1954
21 Moncrieff A Recurrent Neutropenia Arch Dis Childhood 26 438 1951
22 Page A R and Good R A Studies on Cyclic Neutropenia a Clinical and Experimental Investigation A M A J Dis Child 94 623 1957

23 Payne R. Leukocyte Agglutinins in Human Sera Correlation Between Blood Transfusions and Their Development A M A. Arch Int Med 99 587 1957
23a. Payne R. and Rolfs M R Fetomaternal Leukocyte Incompatibility J Clin Invest 37 1756 1958
24 Reimann H A and de Bernardinis C T Periodic (Cyclic) Neutropenia, an Entity a Collection of Sixteen Cases Blood 4 1109 1949
25 Salmonsen L Granulocytopenia in Children, Acta paediat 35 169 1948
26 Sandella J F Cyclic Acute Agranulocytosis Report of a Case With Improvement After Splenectomy Ann Int Med 35 1365 1951
27 Spaet T H and Dameshek, W Chronic Hypoplastic Neutropenia Am J Med 13 35 1952
28 Stefanini, M Mele R. H and Skinner D Transitory Congenital Neutropenia a New Syndrome Am J Med 25 749 1958
29 Sturgis C C Syndromes Associated With Leukopenia a General Review of the Subject (Trimble Lecture) Clinics 1 492, 1942
30 Tullis J L Prevalence Nature and Identification of Leukocyte Antibodies New England J Med. 258 569 1958
31 *Vahlquist, B C and Anjou H Granulocytopenia Chronic Benign*, Acta haemat 8 149 1952
32. Wiseman B K. and Doan C A Primary Splenic Neutropenia Ann Int. Med 16 1097 1942
33 Woodward D J and Solomon, J D Fatal Agranulocytosis Occurring During Promazine (Sparine) Therapy J.A M.A 162 1308 1956

CHAPTER 20

Leukocytosis, Leukemoid Reactions, and Lymphocytosis

LEUKOCYTOSIS

Leukocytosis is the term used to designate an increase in the total number of white blood cells Although such increase may be due to a preponderance of any one of the cellular elements most often the term leukocytosis is used simply to imply an increase due to the neutrophils Neutrophilia is the preferable term and differentiates this form of leukocytosis from lymphocytosis monocytosis and eosinophilia

Physiologic Leukocytosis

Leukocytosis is physiologic when it occurs without associated infection or other demonstrable pathologic lesions The high leukocyte count of the newborn infant with maximal values of 38 000 per cubic millimeter on the first day of life is well known The great majority of babies at this time have an average of 10 000 to 25 000 leukocytes per cubic millimeter although much lower counts have been noted The leukocytosis is initially due to a preponderance of polymorphonuclear neutrophils mainly with a single lobed or double lobed nucleus Trilobed forms appear about the ninth day to the second week[7] Wide variations are found both between different infants and between different counts in the same baby A pronounced drop at the end of the third or fourth day with white blood cells averaging 12 000 per cubic millimeter is maintained until the end of the first year After the end of the second week lymphocytes predominate for the remainder of infancy

Strenuous exercise convulsive seizures emotional disorders fear and agitation and pregnancy and labor have been associated with elevated leukocyte counts Whether or not leukocytosis is attributable to digestion has not been demonstrated conclusively [4]

Pathologic Leukocytosis

The extent of leukocytosis or leukopenia in patients with infection has been related to specific factors present in inflammatory exudates[16] According to this

concept the leukocyte level in the blood stream of patients with inflammatory conditions is the resultant of leukocytosis promoting and leukopenic factors respectively

Usually a neutrophilic leukocytosis with a shift to the left of the polymorphonuclear cells eosinopenia and lymphopenia point to infection The reappearance of eosinophils and segmented neutrophilic leukocytes and the appearance of monocytosis indicate the recovery phase of an illness[14]

Acute infections caused by cocci (*staphylococci streptococci pneumococci* etc) are the most common causes of a neutrophilic increase Because of the tendency of the leukocytes to form pus especially in localized areas examination of the white blood cell count of patients with inflammatory conditions serves as a valuable clinical and diagnostic procedure

Neutrophilic leukocytosis occurs in patients with pneumonia scarlet fever rheumatic fever diphtheria diabetic coma azotemia poisoning by chemicals and drugs such as lead mercury and camphor acute hemorrhage malignant neoplasms with metastases to the bone marrow marked tissue destruction such as burns and sudden hemolysis of red cells In patients with dehydration the extent of leukocytosis is gauged by comparison with elevation of the hemoglobin level and red cell count

Because of the great lability of the hematopoietic system in early life the white blood cell response in younger patients may be disproportionate to the intensity of stimulation Marked leukocytosis and an outpouring of immature cells into the peripheral blood may result from a moderate infection In general excessively high white blood cell counts or leukopenia associated with a shift to the left of the polymorphonuclear cells and toxic granulation indicates a serious outlook, especially if accompanied by excessive numbers of immature white cells and an absence of eosinophils

LEUKEMOID REACTIONS

Leukocytosis refers to white blood cell counts above the normal high of 11 000 per cubic millimeter and leukopenia refers to counts under 4 000 per cubic millimeter Leukemoid reactions are unusual blood responses[1-] associated with exaggerated leukocytic reactions (approximately 50 000 per cubic millimeter and above) or the presence of immature white cells suggesting *leukemia regardless of the leukocyte level* without postmortem evidence of leukemia Depending upon the cell type involved leukemoid reactions may be classified as granulocytic (myeloid) lymphocytic and monocytic Most commonly especially in younger patients a leukemoid condition implies a hyperleukocytosis with a marked shift to immature neutrophilic granulocytes myelocytes metamyelocytes occasional promyelocytes and myeloblasts This response in infants is frequently associated with normoblasts in the peripheral blood It is differentiated from leukemia by the absence of anemia thrombocytopenia hemorrhagic phenomena, splenomegaly and lymphadenopathy and by the infrequent appearance of blasts[9] Exceptionally many of these features may be present in leukemoid reactions[10] The clinical course the transient period of the reaction without recurrence bone marrow aspiration and the absence of tissue infiltration

usually rule out leukemia The leukemoid process also lacks the characteristic gap or hiatus leukemicus between the blast forms and polymorphonuclear neutrophils with few or no transition forms such as myelocytes

Another method of differentiation is by the measurement of alkaline phosphatase activity by histochemical and biochemical methods A high cellular activity of this enzyme is found in the neutrophilic granulocytes of normal persons and of those with leukemoid reactions due to pyogenic infection and negligible amounts are found in the leukocytes of those with chronic myelogenous leukemia[11, 6] This enzymatic activity is also found to be consistently very low in paroxysmal nocturnal hemoglobinuria Low values are also found in patients with a variety of hematologic and nonhematologic diseases such as idiopathic thrombocytopenic purpura myeloid metaplasia infectious mononucleosis pernicious anemia in relapse collagen diseases and refractory or aplastic anemia[4a]

Hyperleukocytosis of the myeloid type occurs following pyogenic infection metastatic carcinoma of bone ulcerative colitis severe hemorrhage primary *tuberculous infection in childhood and acute tuberculosis at any age period* and in episodes of acute hemolysis in the course of an established chronic hemolytic anemia or following infection or drug therapy Congenital leukemia may be difficult to differentiate from the leukemoid reaction occurring with sepsis in the newborn infant A positive diagnosis of leukemia often cannot be made from the blood and bone marrow findings alone and evidence of organ infiltration with white cell precursors is necessary

A leukemoid reaction with a predominance of lymphocytes occurs in patients with whooping cough chickenpox and acute infectious lymphocytosis In all of the patients with these conditions the majority of lymphocytes are normal with small numbers of younger forms with deep blue cytoplasm In patients with infectious mononucleosis the increase in total leukocytes is seldom more than 20 000 per cubic millimeter Elevations above this number are exceptional but the presence of diagnostic atypical lymphocytes readily separates this disease from leukemia

Blood pictures similar to those of patients with myelocytic and lymphocytic leukemia[6] have been reported in patients with miliary tuberculosis A leukemoid reaction with monocytosis may occur in patients with tuberculosis in childhood with a hematogenous spread A count of 82 000 white blood cells per cubic millimeter with 44 per cent monocytes has been reported in a 16 year-old boy with generalized tuberculous adenitis[4]

LYMPHOCYTOSIS

There are few conditions in which a significant increase in lymphocytes occurs so that they are responsible for a moderate or marked leukocytosis An absolute lymphocytosis is found in patients with acute and chronic (nonspecific) infectious lymphocytosis infectious mononucleosis and pertussis and to a lesser extent in those with syphilis tuberculosis and hyperthyroidism A relative increase in lymphocytes is common in patients with conditions with a decreased number of granulocytes A relative lymphocytosis is associated with the leukopenia of measles German measles exanthema subitum and brucellosis It will

be recalled that following the first week of life lymphocytes normally predominate until the fourth or fifth year of life and a lymphocytosis occurring in this period must be evaluated from this standpoint Occasionally in infancy a marked lymphocytosis transiently accompanies an infectious or postinfectious state which cannot be assigned to a specific cause

Acute Infectious Lymphocytosis

Definition Acute infectious lymphocytosis[1-3] a specific disease entity of unknown etiology which is both infectious and contagious It may occur sporadically or in epidemic form The incubation period has been estimated to be between twelve and twenty one days It is characterized by a hyperleukocytosis due to an increase in small mature lymphocytes in which the elevated blood levels persist for approximately two to seven weeks It is a benign infection distinct from infectious mononucleosis acute lymphoblastic and chronic lymphocytic leukemia and miscellaneous infections associated with a lymphocytosis The clinical signs and symptoms may be so mild as to escape attention or the onset may be marked by varying degrees of constitutional reaction A noteworthy feature is the absence of lymphadenopathy and enlargement of the spleen

Age The highest incidence is in children from 1 to 14 years of age with most of the recorded cases occurring during the first ten years of life Several cases have been reported in young adults[4] In general the height of the leukocyte count tends to vary inversely with the age of the patient

Etiology Attempts to identify a causative agent have thus far met with inconclusive results Organisms obtained from routine nasopharyngeal cultures probably represent normal inhabitants or secondary invaders They do not differ from the flora of patients exhibiting the usual hematologic response with similar respiratory infections A bacterial or viral etiology has been postulated[13] but not confirmed

Epidemiology The disease occurs sporadically as multiple cases in families and in institutional epidemics[1, 1, 16, 19, 1] It is therefore contagious but of a low degree of infectivity It has been reported in North Central and South America Europe and Africa In the epidemic reports the majority of patients were devoid of positive physical findings According to some reports the patients presented themselves with signs of respiratory infection or gastrointestinal distress principally diarrhea[19] In the epidemics the peak white blood cell counts in the individual patients ranged from 15 100 to 147 000 per cubic millimeter and the lymphocytes ranged from 63 to 97 per cent

Pathology Microscopic examination of lymph nodes in the few patients studied[3] revealed the striking proliferation of the lining reticuloendothelium with almost complete blockage of the sinuses by masses of these cells and degeneration of the lymph follicles

Clinical Features The condition at times may be so mild as to escape attention or the onset may be marked in individual patients by varying degrees of constitutional reaction Fever upper respiratory infections skin rashes abdominal complaints and meningoencephalitic manifestations may be present Accidental discovery as the result of routine blood examinations is not uncommon par

ticularly in institutional epidemics No consistent correlation is evident between the degree of leukocytosis and the severity of symptoms

Nasopharynx A feature common to most of the patients in whom any manifestations were present is mild infection of the upper respiratory tract The throat may be deeply injected at the time of the initial examination and a history of recent infection of the upper respiratory tract frequently is elicited

Nervous System Increasing experience has shown that acute infectious lymphocytosis should be considered in the diagnosis of an acute febrile illness in which there are symptoms suggestive of central nervous system involvement Headache irritability vertigo, and pain and slight stiffness of the back of the neck may occur without other signs of meningitis The spinal fluid may show a slight pleocytosis with variations in the type of predominating cell[25] although a slight increase in lymphocytes has been reported when the spinal fluid count was positive This disease may simulate poliomyelitis[18] since headache stiffness of the neck restlessness fever, malaise vomiting sore throat and identical spinal fluid changes may occur at the onset of both conditions The blood counts readily separate both diseases Differentiation between these conditions assumes particular importance when the symptoms occur during the summer and early fall

Skin A generalized morbilliform eruption and less commonly a herpetic eruption have been observed in patients during the first week of the disease

Abdomen Signs may be sufficiently pronounced to suggest an acute surgical condition with an elevated temperature vomiting and severe abdominal pain[3] Diarrhea was the most prominent symptom in sixteen of twenty eight patients in one epidemic[18]

Spleen and Lymph Nodes An important diagnostic criterion of infectious lymphocytosis is absence of significant enlargement of the spleen and lymph nodes Pre existing cervical nodes especially of the posterior chain are usually sequelae of previous attacks of nasopharyngitis and are not directly related to the development of acute infectious lymphocytosis Lymphadenopathy and enlargement of the spleen are absent unless they existed prior to the onset of the disease

Incubation Period Because the abnormal white cell counts are unexpectedly encountered in the course of routine blood examinations the exact onset and duration of the leukocytic changes are unknown It is therefore difficult to designate a precise incubation period From the available cases thus far reported it has been possible to fix the approximate length of the incubation period between twelve and twenty one days

Laboratory Findings Highly significant features of diagnostic import are observed among the laboratory findings

Blood The red cells hemoglobin content platelets and sedimentation rate are normal if the disease is uncomplicated When the constitutional reaction is severe the hemoglobin content and the red blood cell count may drop to the lower limit of normal The absence of anemia is an important diagnostic feature in differentiating this ailment from acute lymphoblastic and chronic lymphatic

leukemia and from the lymphocytosis occurring in prolonged postinfectious states No deviations in bleeding or clotting time have been observed

The outstanding feature in patients with acute infectious lymphocytosis

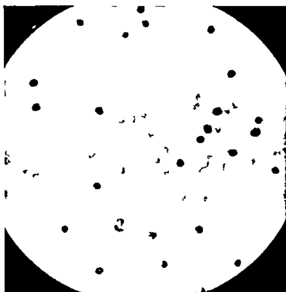


Fig 32 Typical blood smear from a patient with acute infectious lymphocytosis (×400)

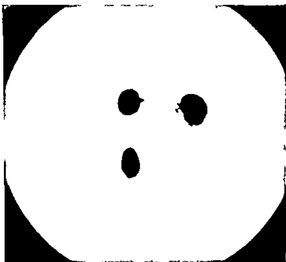


Fig 33 Higher magnification of Fig 32 (×1000) showing that lymphocytes are mainly of the small variety and of uniform size and structure Only occasionally are slightly larger or intermediate types encountered but these are also mature (From Smith C H Acute Infectious Lymphocytosis a Specific Infection J A M A 125 342 1944)

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is the hyperleukocytosis with the relative and absolute increase in normal small mature lymphocytes White blood cell counts at the height of the disease are usually over *40 000 to 50 000* per cubic millimeter with a maximum recorded level over 100 000 per cubic millimeter In the reported institutional epidemics the peak white blood cell counts in the individual patients ranged from 15 100 to 147 000 per cubic millimeter and lymphocytes from 60 to 97 per cent [1] The lymphocytes are mainly the small variety and are of uniform size and normal structure Only occasionally are slightly larger or intermediate types encountered but these are also mature cells The nucleus in these cells is condensed and possesses the coarse chromatin masses of the normal mature lymphocyte These lymphocytes have also been referred to as overripe and have been described as being smaller than normal with a dark purple chromatic material in the nucleus and with very little cytoplasm [17]

The duration of the markedly abnormal leukocytic reaction is usually three to five weeks and occasionally seven weeks An elevation in the percentage of eosinophilic leukocytes occurs frequently during the course of the disease usually at or following the peak of leukocytosis

Bone Marrow The myeloid elements and nucleated red cells are normal in number In many patients the bone marrow shows an increase in the total number of nucleated cells and in the percentage of small mature lymphocytes otherwise it is not abnormal

Heterophil Agglutination (Paul Bunnell) Test The heterophil agglutination test is uniformly negative

Differential Diagnosis (Table 15) Lymphocytic reactions in childhood are difficult to interpret because a predominance of lymphocytes and a greater lability of the blood forming mechanism are common to this age period In the differentiation of acute infectious lymphocytosis these physiologic hematopoietic responses as well as certain specific conditions in which the lymphocytes or their precursors are known to be increased must be evaluated

Infectious Mononucleosis Infectious mononucleosis is usually more severe and characterized by fever sore throat rash rarely jaundice enlargement and tenderness of the lymph nodes and often splenomegaly The febrile phase lasts for one to three weeks but enlargement of the glands and spleen may persist Acute infectious lymphocytosis on the other hand may run an asymptomatic course or there may be transient sore throat fever and constitutional symptoms Lymph nodes and spleen are not enlarged The heterophil antibody reaction is usually positive in patients with infectious mononucleosis but is consistently negative in those with acute infectious lymphocytosis

The blood picture constitutes the most important differential feature In patients with infectious mononucleosis the total white blood cell count usually does not *exceed 20 000 per cubic millimeter* whereas acute infectious lymphocytosis is characterized by a hyperleukocytosis with maximal levels frequently exceeding 50 000 cells per cubic millimeter A more important feature of a hematologic differentiation rests in the morphologic appearance of the lymphocytes *in the two conditions In patients with acute infectious lymphocytosis the* hyperleukocytosis is associated with a preponderance of small lymphocytes

*Table 15 Differential Diagnosis of Acute Infectious Lymphocytosis**

	Acute Infectious Lymphocytosis	*Infectious Mononucleosis*	*Acute Lymphoblastic (Stem Cell) Leukemia*	*Chronic Lymphocytic Leukemia*
Age (usual incidence)	First decade	First 3 decades	First and second decades	After 45 years
Fever and systemic symptoms	Occasionally present	Usually present	Variably present	Infrequent
Enlarged lymph nodes	Absent	Present	Present	Present
Splenomegaly	Absent	Present in 50%	Present	Present
Leukocytosis	Extreme	Moderate	Leukopenia to marked	Usually pronounced
Lymphocytosis	Present	Present	Present	Present
Type of lymphocyte diagnostic cell	Normal small	Atypical abnormal	Lymphoblasts	Mature small
Anemia	Absent	Rare	Present	Late in course
Thrombocytopenia	Absent	Rare	Present	Present
Bone marrow lymphocytes	Increased in number normal small	Occasionally atypical	Lymphoblasts predominate	Lymphocytes predominate
Heterophil agglutination	Negative	Positive	Negative	Negative
Prognosis	Uniformly favorable	Favorable with few exceptions	Uniformly fatal	Uniformly fatal

* From Smith C H Advances in Pediatrics vol 2 Chicago 1947 Year Book Publishers Inc p 64

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possessing a normal cytologic appearance In those with infectious mononucleosis however the distinctive feature is the presence of characteristic atypical mono nuclear cells The variability of atypical lymphocytes and monocytes in persons with this disease contrasts sharply with the uniform size and normal structure of the cells in those with acute infectious lymphocytosis

Acute Lymphoblastic (Stem Cell) Leukemia The extreme leukocytosis and the predominance of lymphocyte in patients with acute infectious lymphocy tosis have occasionally led to the erroneous diagnosis of acute lymphoblastic leukemia Identification of the blast cell which is invariably found in a careful search of the blood smear of patients with leukemia is the distinguishing feature The size of these cells varies from that of a small lymphocyte to twice that size Lymphoblasts are usually round large and uniform in size and the narrow zone of cytoplasm is basophilic and occasionally contains a few large vacuoles

Even more distinctive is the nuclear pattern In contrast with the masses of dense basichromatin found in the nucleus of the normal and large lymphocyte of the patient with acute infectious lymphocytosis the nuclear chromatin of the lymphoblast stains lightly is finely granular stippled or sievelike and nu cleoli are present In patients with acute leukemia with a hyperleukocytosis of the magnitude found in those with acute infectious lymphocytosis the blood exhibits not only large numbers of lymphoblasts but also a severe anemia and a decrease in platelets and the spleen and lymph nodes are enlarged

Examination of the bone marrow also serves to differentiate the two diseases In acute lymphatic leukemia in children bone marrow aspiration usually shows complete replacement of the bone marrow by blast or stem cells In children with acute infectious lymphocytosis on the other hand the myeloid and ery throblastic elements are present in normal proportions and the chief abnormal ity in many patients consists of an increased percentage of normal lymphocytes

Chronic Lymphocytic Leukemia The blood picture of patients with acute infectious lymphocytosis resembles that of those with chronic lymphocytic leukemia in respect to the white blood cells In patients with either of these diseases there is a hyperleukocytosis with a preponderance of small mature lymphocytes and the bone marrow shows increased percentages of normal small lymphocytes and is cellular The age incidence of the two diseases however is very different since chronic lymphocytic leukemia is a disease of older persons In patients with chronic lymphocytic leukemia the spleen and lymph nodes are enlarged anemia and thrombocytopenia eventually develop and the outcome is uniformly fatal in contrast to the negative physical and hematologic findings and favorable prognosis in patients with acute infectious lymphocytosis

Leukemoid Reactions The hyperleukocytosis of infectious lymphocytosis can not be regarded as a leukemoid reaction of the lymphoid type The absence of immature and atypical cells in the blood and bone marrow precludes this desig nation Moreover the leukemoid reaction resulting from infection is transitory the lymphocytic response in patients with acute infectious lymphocytosis is pro tracted Pertussis as a cause of the extreme leukocytosis and lymphocytosis can be excluded by the absence of cough and characteristic clinical manifestations

Miscellaneous Infections A number of specific infections are associated with

lymphocytosis Typhoid fever brucellosis and tuberculosis should be excluded by appropriate tests

Available data render invalid the view that the lymphocytosis represents a peculiar individual response the so called constitutional lymphatic reaction Patients with acute infectious lymphocytosis afflicted with antecedent con current, or subsequent illnesses such as acute otitis media or pneumonia ex hibit a neutrophilic response

Treatment and Prognosis There is no specific therapy Treatment is sympto matic and similar to that in patients with other acute infections of the upper respiratory tract Because of the low infectivity of the condition the large number of asymptomatic cases and the brief span of constitutional reactions in the febrile cases no isolation seems necessary The prognosis in all cases has been uniformly excellent and no sequelae have been observed

Chronic Nonspecific Infectious Lymphocytosis (Low Grade Fever Syndrome)

Clinical Picture The symptom complex of chronic nonspecific infectious lymphocytosis is characterized by a persistent slight to moderate leukocytosis and a preponderance of lymphocytes It is frequently encountered in pediatric practice and is distinct from acute infectious lymphocytosis Difficulties in diagnosis arise when an acute infection of the upper respiratory tract is followed for prolonged periods by a low grade fever with the temperature ranging from 99 F up to and usually not including 101 F In addition to the protracted fever the symptom complex includes anorexia pallor irritability fatigability and abdominal pain localized to the region of the umbilicus The fauces are injected the tonsils when present are usually greatly hypertrophied and postnasal dis charge is frequent At times the superficial cervical lymph nodes are slightly en larged The heart and lungs are normal there are no murmurs or other cardiac ab normalities In infants and young children the spleen may be palpable but this finding is inconstant especially in older children The age of incidence is usually from 6 months through 10 years especially in the period from 3 to 6 years Cases are less commonly observed between 3 and 6 months of age In these infants moderate elevations of temperature (103 to 104 F) frequently interrupt the usual course of low grade fever (temperature up to 101 F) Although these episodes are usually related to exacerbations of infection of the naso pharynx other causes of lymphocytosis must be eliminated

Blood The accompanying slight to moderate leukocytosis and lymphocytosis usually persist for periods of months and sometimes a year or more The total white blood cell counts usually range from 8 000 to 18 000 rarely reaching 20 000 per cubic millimeter with 60 to 80 per cent lymphocytes White blood cell counts reaching 25 000 per cubic millimeter are frequently observed in infants under 6 months of age The hemoglobin ranges between 10 and 11 gm per 100 ml and the platelets are normal in number Lymphocytosis in this group of pa tients with chronic disease resembles that which occurs in postinfectious states in infants and children Most of the lymphocytes are of the small mature type struc turally similar to those in patients with acute infectious lymphocytosis Oc

casionally cells of the larger variety are seen which are of normal shape and which possess a deeply basophilic cytoplasm and an eccentric nucleus which stains more intensely than that of the normal lymphocyte The same depth of staining is observed in another type of lymphocyte which is about one and one half times the size of a red cell and whose nucleus is round oval or slightly indented In infants the zone of cellular cytoplasm is often wider and the basophilia deeper than in the corresponding cells of blood in older children These larger cells are not specific since they appear in increased numbers in the blood of young children during the active stage and convalescence of a large variety of infections and may occasionally be found in the blood of normal infants

Differential Diagnosis Chronic nonspecific infectious lymphocytosis is not contagious and does not appear in epidemic form in contrast to acute infectious lymphocytosis Moreover the acute disease is usually asymptomatic the blood reaction is comparatively short and well defined hyperleukocytosis is marked and the disease is not characterized at any age period by either lymphadenopathy or splenomegaly

Chronic nonspecific infectious lymphocytosis is often confused with infectious mononucleosis especially in young children acute lymphoblastic leukemia and acute rheumatic fever Not only is the heterophil agglutinin test negative in the patient with chronic infectious lymphocytosis but the lymphocytes lack the irregularity of shape and the abundant frequently vacuolated, and foamy cytoplasm which are distinctive features of the cells of patients with infectious mononucleosis

Acute lymphoblastic (stem cell) leukemia is differentiated by the presence of lymphoblasts hyperleukocytosis often leukopenia anemia (hemoglobin below 10 gm per 100 ml) and thrombocytopenia Marked lymphadenopathy and splenomegaly are additional confirmatory features but may be absent in the patient with early disease Examination of the bone marrow serves to differentiate the two diseases unequivocally although this procedure is rarely necessary

Many of the patients are referred with a diagnosis of acute rheumatic fever because of a more or less continuous low grade fever a failure to gain weight or loss of weight fatigability and abdominal pain From the hematologic standpoint acute rheumatic fever is usually associated with moderate leukocytosis with polymorphonuclear leukocytes as the predominant cell in contrast to chronic nonspecific infectious lymphocytosis in which lymphocytosis characterizes the blood smear The sedimentation rate is markedly increased in patients with rheumatic fever and does not approach normal until some time after the fever and clinical signs have abated whereas in those with chronic lymphocytosis the sedimentation rate is normal or only slightly increased for short intervals Nosebleeds the development of carditis pains in the arms legs and joints subcutaneous nodules and erythematous rashes are other features present in patients with rheumatic fever and absent in those with lymphocytic diseases

A disease identical with or similar to chronic infectious lymphocytosis has been reported as lymphocytic fever [6] It occurs in children chiefly under 2 years of age with enlargement of the liver and spleen slight lymphadenopathy

and a leukocytosis with a preponderance of small lymphocytes persisting for months and occasionally a year or more

The lymphocytic blood picture in patients with chronic infectious lymphocytosis is a useful diagnostic aid in differentiating the abdominal pain which may occur in both diseases from surgical conditions and from nonspecific mesenteric lymphadenitis which are associated with a neutrophilic response

Treatment and Prognosis Chronic nonspecific infectious lymphocytosis may be a source of anxiety to both parents and physician It is therefore desirable to emphasize that the prognosis is entirely favorable that treatment is symptomatic and that subsidence of the disease depends upon relief of infection in the nasopharynx Striking improvement often follows tonsillectomy but tonsillectomy is not advocated as a routine measure

Repeated temperature readings are unnecessary and antibiotics are futile in altering the pattern of low grade fever Once the diagnosis is established restriction of activities is unnecessary

REFERENCES

1 Barnes G R Jr Yannet H and Lieberman R A Clinical Study of an Institutional Outbreak of Infectious Lymphocytosis Am J M Sc 218 646 1949
1a Beloff J S and Gang K M Acute Poliomyelitis and Acute Infectious Lymphocytosis Their Apparent Simultaneous Occurrence in Summer Camp J Pediat 26 586 1945
2 Custer R P An Atlas of the Blood and Bone Marrow Philadelphia 1949 W B Saunders Co
3 Duncan P A Acute Infectious Lymphocytosis Am J Dis Child 66 267 1943
4 Duncan P A Acute Infectious Lymphocytosis in Young Adults New England J Med 233 177 1945
5 Finucane D L and Philip R S Infectious Lymphocytosis Am J Dis Child 68 301 1944
6 Gardner R H and Mettier S Lymphocytic Leukemoid Reaction of the Blood Associated With Miliary Tuberculosis Blood 4 767 1949
7 Carrey W E and Bryan W R Variations in White Blood Cell Counts Physiol Rev 15 597 1935
8 Gibson A Monocytic Leukemoid Reaction Associated With Tuberculosis and a Mediastinal Teratoma J Path & Bact 58 469 1946
9 Hill J M and Duncan C N Leukemoid Reaction Am J M Sc 201 847 1941
10 Hilts S V and Shaw C C Leukemoid Blood Reaction New England J Med 249 434 1953
11 Koler R D Seaman A J Osgood E E and Vanbellinghen P Myeloproliferative Diseases Diagnostic Value of the Leukocyte Alkaline Phosphatase Test Am J Clin Path 30 295 1958
12 Krumbhaar E B Leukemoid Blood Picture in Various Clinical Conditions Am J M Sc 172 519 1926
13 Landolt R F Akute infektiöse Lymphocytose im Kindesalter Helvet paediat acta 2 377 1947
14 Lawrence J S Adams W S and Valentine W N White Blood Changes in Clinical Disorder J A M A 150 454 1952
15 Lemon B K and Kaump D H Infectious Lymphocytosis Report of Epidemic in Children J Pediat 36 61 1950
16 Merkin V Leukocyte Level in Blood Stress With Inflammation Blood 4 1323 1949
17 Meyer I M Acute Infectious Lymphocytosis Am J Clin Path 16 244 1946
18 Pettman M G Kaster J D Geelt L A and Lembert G L Epidemic of Acute Infectious Lymphocytosis With Diarrhea Pediatrics 3 214 1949

19 Reyersbach G and Lenert T F Infectious Mononucleosis Without Clinical Signs or Symptoms Am J Dis Child 61 237 1941
20 Rosenbaum S Febris Lymphocytotica Ann paediat 152 117, 1938
21 Scalettar H E Maisel J E and Bramson M Acute Infectious Lymphocytosis A M.A Am J Dis Child 88 15 1954
22 Smith C H Infectious Lymphocytosis Am J Dis Child 62 231 1941
23 Smith C H Acute Infectious Lymphocytosis Specific Infection Report of Four Cases Showing Its Communicability J A M A 125 342 1944
24 Sturgis C C and Bethell F H Quantitative and Qualitative Variations in Normal Leukocytes Physiol Rev 23 279 1943
24a Tanaka K R Valentine W N and Fredricks R E Disease or Clinical Conditions Associated With Low Leukocyte Alkaline Phosphatase New England J Med 262 912 1960
25 Thelander H E and Shaw E B Infectious Mononucleosis With Specific Reference to Cerebral Complications Am J Dis Child 61 1131 1941
26 Wiltshaw E and Moloney W C Histochemical and Biochemical Studies in Leukocyte Alkaline Phosphate Activity Blood 10 1120 1955

CHAPTER 21

Infectious Mononucleosis

Definition Infectious mononucleosis is an acute infectious disease of unknown etiology It is usually benign has a self limited course and is characterized by lymphocytosis with atypical lymphocytes in the peripheral blood enlarged lymph nodes and spleen and various other constitutional symptoms In a large number of patients the serum reveals a high titer of agglutinations for sheep red cells

Historical The earliest descriptions of the disease were by Filatov[11] in 1885 and by Pfeiffer[3] in 1889 who gave it the name glandular fever The latter reported a symptom complex in children marked by an acute onset and enlargement of the posterior cervical nodes the liver and spleen Case reports of glandular fever appeared in the literature in increasing numbers emphasizing the clinical aspects of the disease In 1920 Sprunt and Evans[11] drew attention to its infective nature described the changes in the peripheral blood characterized by an increase in mononuclear and other normal cells and designated the disease as infectious mononucleosis Soon afterward Longcope[27] and Downey and McKinlay[9] provided an accurate morphologic description of the cells diagnostic of this disease The three types of cells described by the latter are frequently referred to as Downey cells Tidy and Daniel at this time[45] confirmed the significance of these cells and pointed out that glandular fever and infectious mononucleosis were identical conditions From that time until the serologic studies were made this disease received increasing recognition and both the clinical features and blood picture became more sharply defined

A basic contribution in the diagnosis of infectious mononucleosis was made in 1932 with the discovery by Paul and Bunnell[30] that the serum of patients with this disease agglutinated the washed erythrocytes of sheep in concentrations above a normal titer These agglutinins are termed heterophil antibodies because the reaction is with antigens of a different species that has nothing to do with their development—in this case sheep cells This discovery stimulated renewed *interest in this disease particularly in its immunologic aspects* and provided a diagnostic criterion of great accuracy in a disease with such protean manifestations

Pathology The focal cellular and perivascular infiltration with normal and

abnormal lymphocytes in so many of the organs such as the liver nervous system kidneys heart and lungs accounts in large measure for the variety of clinical symptoms

Atypical lymphocytes are not present in the bone marrow although a shift to the left of polymorphonuclear neutrophils with increased numbers of myelocytes occasionally is observed In the lymph nodes the nodal architecture is maintained and marked proliferative activity of the pulp with packing by lymphocytes is present Throughout the pulp in the sinuses and on the edges of the germinal centers large numbers of the atypical mononuclear cells observed in patients with infectious mononucleosis have been described [14]

Clinical Features The problem of diagnosis usually is resolved by combining the heterophil antibody test with the hematologic and clinical findings especially since seronegative cases of this disease have occurred In early stages of the disease the diagnosis is puzzling since enlarged and tender lymph nodes characteristic blood abnormalities and a positive heterophil test often do not appear until the end of the first week or ten days

In recent years also the involvement of the liver and to a lesser extent the peripheral and central nervous systems has been reported with sufficient frequency to serve as an accessory diagnostic feature The causative agent has not yet been identified although a virus etiology is suspected [0] The disease appears in both epidemic and sporadic forms It is infectious but manifests a low degree of contagiousness It occurs at any age most commonly in children and young adults and rarely in infants The incubation period is given as four to fourteen days

The disease is marked by fever sore throat and epistaxis frequently with acute tonsillitis and often with generalized enlargement of the lymph nodes and spleen The edge of the spleen is soft and the organ is never greatly enlarged The spleen is palpable in approximately 50 per cent of patients Other clinical features to attract attention include headache vomiting urticaria rash jaundice periorbital edema and pain referable to the external muscles of the eyes Abdominal pain may be a predominant feature and the signs and symptoms may simulate acute appendicitis Appendectomies have been performed before the true nature of the condition was recognized [43] Hematuria and rectal bleeding have been encountered[45] but are rare in children

In a few instances an enanthem consisting of pinhead sized spots which darken in three to four days appears on the palate True petechiae numbering ten to several hundred clustered near the junction of soft and hard palate and more posteriorly close to the midline have also been described [38] They appear three days to ten weeks after the onset of symptoms and last three to eleven days These have not been associated with a hemorrhagic diathesis

An exanthem occurs more frequently The rash may be macular maculopapular purpuric or morbilliform resembling rubella Rose spots simulating those found in patients with typhoid fever have also been observed Falsely positive serologic reactions for syphilis [14] also have been encountered occasionally in the first week of the disease but revert to negative between the third and tenth weeks In association with a rubelliform rash and lymphadenopathy differentia

tion of syphilis from infectious mononucleosis may be difficult in the early weeks

Clinical Types Although symptoms vary widely they have been classified by some authors into several clinical categories The glandular or childhood type is marked by sore throat fever and generalized lymphadenopathy and frequently *occurs in epidemics In young adults the pharyngeal type is common and is* marked by fatigue and sore throat general malaise low grade fever and slight to moderate enlargement of lymph nodes In patients with the febrile type the onset is sudden often with prolonged typhoidlike fever headaches chills and prostration followed later by rash and lymph node and splenic enlargement Less commonly the onset is ushered in variously as an acute allergic reaction with angioneurotic edema jaundice the signs of meningoencephalitis or a severe angina with a membrane on or near the tonsils which mimics diphtheria

In some children the initial period of illness extending for five to seven days may be marked by the temperature reaching levels of hyperpyrexia with no other abnormal physical signs than a slightly palpable spleen There may be no *complaints Leukocytes range from 5 000 to 6 000 per cubic millimeter* with a predominance of polymorphonuclear neutrophils After several days of fever a diffuse eruption resembling rubella may appear without the enlargement of the postauricular postcervical and occipital lymph nodes which marks this disease At the end of a week temperatures are lower eruptions are fading and a moderate leukocytosis with a predominance of lymphocytes and a substantial number of diagnostic cells of infectious mononucleosis is noted Throughout the entire extent of the disease symptoms and physical signs may be minimal

In rare instances the early stages of infectious mononucleosis simulate leu*kemia both clinically and hematologically and careful scrutiny of blood smears* and heterophil antibody tests is needed to differentiate between the two conditions Of the clinical features that have been enumerated the presence of unexplained fever posterior cervical adenopathy pharyngitis and a tonsillar exudate should prompt a careful examination of the blood smear for the atypical cells found in patients with infectious mononucleosis

Involvement of the Nervous System The pathologic changes in patients with infectious mononucleosis are widespread and include changes in the central nervous system in the liver and to a lesser extent in the heart and spleen In*volvement of the nervous system has been increasingly recognized in children* as well as in adults It has been stated [1] that this disease should be considered in the differential diagnosis of encephalitis and meningitis for which no specific cause has been found The nervous system manifestations vary from intense headache blurred vision and stiffness of the neck to definite evidence of central nervous system involvement Encephalitis encephalomyelitis serous meningitis acute polyradiculoneuritis as observed in patients with the Guillain Barre syndrome facial palsy retrobulbar neuritis and other signs of isolated or diffuse upper and lower motor neuron lesions have been described Although the association of *seizures with infectious mononucleosis is infrequent this disease should* be considered in the differential diagnosis of unexplained and ill defined convulsive episodes [4] Alterations in the spinal fluid may occur to the extent of a moderate pleocytosis increased pressure increase in mononuclear cells and less frequently

increased protein Neurologic complications appear two to four weeks after the onset of infectious mononucleosis but may precede or occur concomitantly with it Recovery from the neurologic complications is the rule although permanent damage and even death from respiratory paralysis have been noted [8]

Involvement of the Liver Increasing evidence supports the view that impaired liver function accompanies the majority of cases of this disease Jaundice may occur as the initial symptom or later in the disease with enlargement of lymph nodes More frequently hepatitis in the absence of jaundice has been reported in many studies as revealed with the use of serial liver function tests In an examination of sixty two children with a presumptive diagnosis of infectious mononucleosis Hsia and Gellis[19] have shown 84 per cent to have abnormal liver function It has been suggested therefore that tests for liver function such as cephalin flocculation thymol turbidity and Bromsulphalein retention tests be used to supplement other diagnostic tests for infectious mononucleosis Similarly in patients suspected of having infectious hepatitis it may be advisable to test for heterophil antibodies Patients especially children with infectious mononucleosis however recover quickly and completely whereas the course of those with infectious hepatitis is more prolonged and complicated The impaired liver function may also account for prolonged convalescence and for the easy exhaustion and weakness found especially in young adults though less commonly in children Pathologic examination of the liver reveals a varying picture—from a normal architecture to focal hepatitis with loss of liver cells cellular infiltration by atypical mononuclear cells dilatation and invasion of sinuses areas of scattered focal necrosis and enlarged portal lymph nodes The usual course of hepatitis is two to six weeks with a rapid return to normal [8]

Involvement of the Heart Cardiac complications occur infrequently especially in childhood and are detected by electrocardiographic and clinical examinations These have been attributed to focal interstitial infiltrations of mononuclear cells and lymphocytes in the myocardium [1] Fatal acute myocarditis with focal microscopic areas of necrosis and fragmentation of heart muscle fibers has been reported [1] Although scattered necropsy reports have shown involvement of the myocardium there is every evidence that valvular heart disease is a rare complication of infectious mononucleosis [17] The changes observed on the electrocardiogram are transient and consist of lowering or inversion of the T waves in various leads and increased P R interval Since clinical examination infrequently reveals an affected heart the correct interpretation of these electrocardiographic findings remains uncertain [18] The first symptoms of infectious mononucleosis may be those of acute benign pericarditis [15 39] Although patients with fatal nervous system involvement or splenic rupture are more likely to show definite myocarditis chronic heart disease is not to be expected as a sequela in patients with infectious mononucleosis

Rupture of the Spleen Rupture of an enlarged spleen can occur spontaneously or as a result of trauma and has also been related to vigorous palpation of the abdomen The liability to rupture is not encountered until the third week of illness and is due to the softness and friability of the spleen resulting from thinning of the capsule and dissolution of the trabeculae by lymphocytic infiltration [4]

Laboratory Findings Characteristic abnormalities are found in the peripheral blood and by serologic examination

Blood Picture In the majority of patients the blood picture constitutes the most important diagnostic feature of infectious mononucleosis The total white blood cell count usually ranges from 10 000 to 20 000 per cubic millimeter during the peak of the disease in the second and third weeks and exceeds the upper limit to 50 000 in rare cases At such extreme levels it must be differentiated from acute infectious lymphocytosis Leukocytosis usually accompanies fever and enlargement of the lymph nodes The count however varies greatly and leukopenia with white blood cell levels as low as 3 000 per cubic millimeter may mark the disease in its first week Frequently in young adults the white blood cell count is normal but clinical symptoms and the blood smear provide the clue to diagnosis

The striking feature in the blood smear is the shift from an early normal or increased percentage of polymorphonuclear neutrophils to a predominance of the mononuclear elements to the extent of 50 to 90 per cent The term mononuclear cells was formerly applied to both monocytes and lymphocytes—hence the term infectious mononucleosis The majority of abnormal cells are now known to be derivatives of the lymphocytic series and must be differentiated from the immature lymphocytes of leukemia The atypical mononuclear cell in the blood of patients with infectious mononucleosis represents its distinctive feature The identification of lymphocytes however has been verified by supravital staining Monocytes may also be increased The characteristic mononuclear cells consisting mainly of lymphocytes are larger than normal their cellular edges are often ragged and irregular and the cytoplasm is usually abundant staining a darker blue with Wright's stain than do normal large lymphocytes The cytoplasm contains fine or coarse azure granules and one of the most striking features is the vacuolation The vacuoles are of two types they may be large and scattered in cells with abundant cytoplasm or they may be smaller and more numerous The combination of fine vacuolation and basophilia gives the cytoplasm a distinctive foamy appearance

The nucleus of the cells in patients with infectious mononucleosis may be round oval or kidney shaped occasionally it is divided and often eccentrically placed The Downey type I cell refers to the cells with dark blue foamy cytoplasm and an irregular kidney shaped nucleus The Downey type II cell possesses larger amounts of light blue cytoplasm and an eccentrically placed nucleus of normal appearance The diagnosis in the acute stages of infectious mononucleosis is made by the presence of one or another of the characteristic types of cells and by the assortment of unusual cells in the blood smear

Occasionally small numbers of large rounded cells with well differentiated clear nuclei and lightly basophilic cytoplasm resembling reticuloendothelial cells appear in the peripheral blood They probably originate from areas of reticulum cell proliferation in affected lymph nodes [10]

Just as agglutination may appear in higher titer in patients with diseases other than infectious mononucleosis so have these atypical cells been noted in patients with a variety of conditions such as infectious hepatitis virus pneumonia German measles, measles acute brucellosis influenza roseola infantum and others

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Fig 54 Infectious mononucleosis *1* Photomicrograph of several types of atypical lymphocytes found in the blood smear of patients with infectious mononucleosis A and C possess a finely vacuolated cytoplasm giving a foamy appearance exaggerated in C by the deep basophilia The color of the cytoplasm with Wright's stain varies from a slate color to the deep blue of a plasma cell The nuclear pattern in *B* is not so coarse as that in the other cells and is more immature with finer strands of lighter staining chromatin Several nucleoli are present This cell resembles somewhat the lymphoblast and is often referred to as the Downey type III cell (see K in Fig 54 *2*) (Courtesy Dr Ralph L Engle Jr New York N Y)

2 Drawings of representative cells in the blood smear of patients with infectious mononucleosis Although a number of different cell types usually appear in the blood smear at one time especially at the height of the disease all of these cells were not found together in any single case *F* is a cell with irregular edges eccentric nucleus abundant cytoplasm and concentration of basophilia in the periphery A lightly staining perinuclear zone is observed in *K* Deep basophilia of the cytoplasm is noticeable in *G H* and *K* Vacuolation of the cytoplasm is present in *E* and *G* The finer vacuoles and deep basophilia in *G* give the cytoplasm a foamy appearance an important diagnostic feature Cells of type K occurred most infrequently The nucleus of the K type cell stains lightly and approaches in immaturity the nucleus of the lymphoblasts (From Smith C H Infectious Lymphocytosis Am J Dis Child 62 231 1941)

Because of the increased incidence in so called virus diseases the atypical lymphocytes have been termed virocytes [3, 4] It is important to point out that the blood smears of young infants are particularly prone to show these cells in a number of nonspecific infections unrelated to infectious mononucleosis In patients with conditions other than infectious mononucleosis however the abnormal mononuclear cells appear in much smaller numbers rarely reaching 10 per cent At the height of the illness this quantitative difference serves as a differential criterion Eosinophilia has been observed during the acute stage of the disease but is even more common during convalescence

It has already been stated that the blood picture in the introductory stages may simulate leukemia This confusion results from the occasional presence of immature cells resembling lymphoblasts (Downey type III cells) especially when they are observed in association with anemia leukopenia and thrombopenia However the eventual appearance of atypical lymphocytes in significant numbers provides an indication of the true nature of the process

The red blood cell count hemoglobin concentration platelet count sedimentation rate coagulation time and bleeding time are usually normal A mild anemia may attend convalescence Exceptionally thrombocytopenic purpura [6, 9] and acute hemolytic anemia[10, 44] have been reported during the course of infectious mononucleosis

Acute hemolytic anemia occurring during the course of infectious mononucleosis is often associated with marked prostration high fever and deepening jaundice The anemia may be of sufficient severity to necessitate splenectomy [4] Uncommonly a positive antiglobulin (Coombs) test [16, 3] cold agglutinins [44] and autohemolysins indicative of an autoimmune mechanism have been present

It has been postulated that the etiologic factors viral or bacterial responsible for causing infectious mononucleosis may also injure the red cells or platelets directly or through antibody formation Abnormal splenic function or hypersplenism may be involved similarly in reducing any of the formed elements of the blood in patients with infectious mononucleosis inasmuch as this disease is so often accompanied by splenomegaly

Heterophil Antibody (Paul Bunnell) Test Although there is no unanimity of opinion a heterophil antibody titer of 1 112 is usually considered diagnostic although the critical level stated in various laboratories ranges from 1 56 to 1 1792 No correlation appears to exist between the heterophil antibody titer and the severity of the disease The heterophil antibody test has been found positive in 60 to 90 per cent of patients and for that reason examination of the blood smear for atypical lymphocytes is still of paramount importance The timing of tests is important since the titer may be low in the first week and usually reaches a peak in the second and third weeks in some instances to extraordinary levels An elevated titer may persist during convalescence usually two to five months sometimes six months and longer after the symptoms subside It may revert to normal however in six to eight weeks Repetition of tests therefore is important in doubtful cases and increased positive reactions probably depend upon this factor When venous blood is difficult to obtain a fingertip microheterophil agglutinin

technique has been described which compares favorably with the regular heterophil technique [16a]

According to Vahlquist and associates,[46] a positive Paul Bunnell reaction is rare below 5 years of age and this is explained by a defect in antibody formation during this period Past this age the reaction does not differ from adult cases with respect to intensity and duration of the serologic reaction

Differential Agglutination Tests Heterophil agglutinins against sheep cells are present in low titer in normal serum and after the injection of horse serum These are the Forssman type of antibodies which are distinct from those elaborated in patients with infectious mononucleosis The fact that the Forssman type of antibody combines actively with guinea pig kidney and does not react with beef red cells is employed in the diagnosis of infectious mononucleosis The heterophil antibodies present in patients with serum sickness and in those with normal serum can be removed by absorption with guinea pig kidney whereas antibodies in the serum of patients with infectious mononucleosis are not absorbed to the same extent Although complete absorption with beef red cells is to be expected in patients with infectious mononucleosis if at least one fourth of the agglutinin titer remains after absorption with guinea pig kidney the test is positive for infectious mononucleosis [6]

Table 16 indicates the scheme of differential agglutination

Table 16 Scheme of Differential Agglutination

Source of Serum	Heterophil Agglutinins After Absorption by	
	Guinea Pig Kidney	Beef Cells
Infectious mononucleosis	Present	Absent
Normal serum	Absent	Present
Serum sickness	Absent	Absent

Absorption tests therefore add to the accuracy of the diagnosis especially in patients with low titers In clinical practice however these tests are frequently not undertaken when the blood smear and supportive clinical features confirm the diagnosis In the presence of other unequivocal evidences of this disease a negative serologic test does not rule out the diagnosis of infectious mononucleosis

Absorption tests are especially pertinent in rare instances in which the Paul Bunnell test has been found positive without the associated features of infectious mononucleosis These include a variety of conditions in which an occasional patient may reveal a significant titer—for example infectious hepatitis purpura German measles scarlet fever sarcomas upper respiratory infections primary atypical pneumonia monocytic and myelogenous leukemia thrombocytopenic purpura [42] and Hodgkin s disease [36] In one series[40] elevated heterophil antibody titers (1 112 or more) were observed in 19 per cent of patients with acute leu

kemia This antibody was completely or almost completely absorbed by guinea pig kidney demonstrating its relationship to the Forssman type in contrast to poor absorption in patients with infectious mononucleosis No elevated heterophil antibodies were observed in this series in patients with chronic leukemia

A sharp reduction in titer has been observed when enzyme (papain) treated sheep erythrocytes are mixed with the serum of patients with infectious mononucleosis In contrast the serum of patients with an elevated heterophil antibody titer unrelated to infectious mononucleosis showed a conspicuous increase in reactivity with enzyme treated sheep red cells as compared with their reactivity with untreated cells

We have noted unabsorbed titers ranging from 1 112 to 1 7168 in patients with thrombocytopenic purpura severe Cooley's anemia lymphoma and cirrhosis of the liver and in children with regional ileitis

Recently an ox cell hemolysin test has been checked against the heterophil antibody test in patients with infectious mononucleosis and a degree of sensitivity was found to justify further study [1]

Differential Diagnosis The diverse clinical picture the wide range of symptoms and the pathologic lesions in patients with infectious mononucleosis may obscure the diagnosis and require a consideration of many diseases which it mimics These include leukemia acute infectious lymphocytosis poliomyelitis German measles chronic upper respiratory infections rheumatic fever acute appendicitis infectious hepatitis pertussis mumps influenza and lymphocytic choriomeningitis Usually the enlargement of the lymph nodes and the spleen sore throat, fever numerous atypical lymphocytes in the blood smear an increased titer of heterophil antibodies and the absence of anemia and of invasive blast cells in the marrow establish the proper diagnosis Although infectious hepatitis and infectious mononucleosis resemble each other by liver function tests and in pathologic findings infectious mononucleosis usually is associated with an increased heterophil antibody titer and the number of patients with clinical jaundice is small

It should also be pointed out that during the first months of life small numbers of lymphocytes occasionally are observed in the blood smears from normal infants which resemble the abnormal cells found in patients with infectious mononucleosis These cells however bear no relationship to this disease or to any other related condition

Prognosis In spite of the alarming nature of many of the symptoms the prognosis is good except in rare patients with splenic rupture or infections Fatal cases of infectious mononucleosis have occurred however without rupture of the spleen They reveal a widespread involvement of lymphoid tissue and the presence of atypical mononuclear cells throughout a majority of the organs[43]

Treatment The disease is self limited and no specific therapeutic measure has proved effective Treatment is symptomatic and reliance must be placed upon rest and supportive measures in view of involvement of various organs especially the liver nervous system and heart

In children complications are rare although the over all incidence in all age groups has been approximated as 5 per cent[43] Antibiotics may be of value in

patients with severe sore throat and in controlling secondary infections Recently ACTH and cortisone have been employed in diminishing the severity of the disease [7] in respiratory distress [33] in relieving allergic manifestations and in the treatment of meningoencephalitis [13] It should be emphasized that the value of the steroid hormones in reducing constitutional symptoms must be balanced against the risk of disseminating a concomitant or occult bacterial infection [9]

For the patients with hemorrhagic manifestations due to thrombocytopenia *treatment with the adrenocortical steroids has been effective Prednisone or pred* nisolone is given in a dosage of 5 to 10 mg every six hours to children under the age of 10 years with a higher dosage ranging to a maximum of 20 mg every six hours in the adolescent patient Treatment for five to seven days should effect an increase in platelets and in the control of hemorrhage [48] In very unusual cases splenectomy is necessary to control hemorrhage due to thrombocytopenia For patients with the rare complication of hemolytic anemia transfusions are given with or without the steroid hormones In one patient in whom cortisone was ineffective splenectomy was resorted to with a favorable response [44]

Recurrences Although unquestionable recurrences have been reported they are extremely rare and frequently fail to demonstrate significant heterophil antibodies However a nonspecific rise in titer occurs some months after the initial attack especially following an upper respiratory infection Since atypical lymphocytes are known to persist for extended periods recurrences may be errone *ously diagnosed More extensive experience is required with a combination of* laboratory techniques especially liver function tests to rule out the influence of hepatitis as a causative factor in prolongation and chronicity of the disease or its recurrence

REFERENCES

1 Allen F H and Kellner A Infectious Mononucleosis an Autopsy Report Am J Path 23 463 1947
2 Angle R M and Alt H L Thrombocytopenic Purpura Complicating Infectious Mononucleosis Report of a Case and Serial Platelet Counts During the Course of Infectious Mononucleosis Blood 5 449 1950
3 *Bakst H and Liebowitz J Infectious Mononucleosis Report of Case With First Appear* ance of Significant Numbers of Heterophil Antibodies and Abnormal Lymphocytes (Virocytes) in Seventh Week of Illness Am J Med 13 235 1952
4 Custer R P and Smith E B The Pathology of Infectious Mononucleosis Blood 3 830 1948
5 Dameshek W and Grassi M A Infectious Lymphadenosis and Thrombocytopenic Purpura Recovery After Splenectomy Blood 1 339 1942
6 Davidsohn L Stern K and Kashiwagi C The Differential Test for Infectious Mononucleosis Am J Clin Path 21 1101 1951
7 Doan J K and Weisberger A J Use of ACTH in Infectious Mononucleosis Ann Int Med 38 1058 1953
8 Dolgopol V B and Husson G S Infectious Mononucleosis and Neurological Complications Report of a Fatal Case Arch Int Med 83 179 1949
9 Downey H and McKinlay C A Acute Lymphadenosis Compared With Acute Lymphatic Leukemia Arch Int Med 32 82 1923
10 Downey H and Stasney J The Pathology of the Lymph Nodes in Infectious Mononucleosis Folia haemat 54 417 1936

11 Filatov N F Lectures on the Acute Infectious Diseases of Children vol 1 Moscow 1885 A Lang p 13
12. Fish M and Barton H R Heart Involvement in Infectious Mononucleosis A M A Arch Int Med 101 636 1958
13 Frankel E P Shiver C B Jr Berg P and Caris T N Meningoencephalitis in Infectious Mononucleosis Report of a Case Treated With Cortisone J A M A 162 885 1956
14 Gall E A and Stout H A The Histological Lesion in Lymph Nodes in Infectious Mononucleosis Am J Path 16 433 1940
15 Gardiner C C Acute Pericarditis as the Initial Manifestation of Infectious Mononucleosis Am J M Sc 237 352 1959
16 Hall B D and Archer F C Acute Hemolytic Anemia Associated With Infectious Mononucleosis New England J Med 249 973 1953
16a Heemstra J G and Stanage W F A Fingertip Microheterophile Agglutination Technic Pediatrics 23 753 1959
17 Hoagland R J Cardiac Involvement in Infectious Mononucleosis Am J M Sc 232 252 1956
18 Houck, A H Involvement of the Heart in Infectious Mononucleosis (editorial) Am J Med 14 261 1953
19 Hsia D Y Y and Gellis S S Hepatic Dysfunction in Infections in Children, A.M.A. Am J Dis Child 84 175 1952
20 Hunt J S The Pathogenesis of Infectious Mononucleosis Am J M Sc 228 83 1954
21 Karpinski F E Jr Neurologic Manifestations of Infectious Mononucleosis in Childhood Pediatrics 10 265 1952
22 Kaufman R E Recurrences in Infectious Mononucleosis Am Pract & Digest Treat 1 673 1950
23 Lazar H P Manfredi R and Hammond J H Seizures in Infectious Mononucleosis Am J Med 21 990 1956
24 Liebowitz J Infectious Mononucleosis Modern Medical Monographs vol 5 New York, 1953 Grune & Stratton Inc
25 Liebowitz S Infectious Mononucleosis Report of a Case Presenting as Acute Anaphylactic Shock Treated With ACTH and Cortisone New York J Med 51 2711 1954
26 Lloyd P C Acute Thrombocytopenic Purpura in Infectious Mononucleosis Am J M Sc 207 620 1944
27 Longcope W T Infectious Mononucleosis (Glandular Fever) With a Report of 10 Cases Am J M Sc 164 781 1922
27a Muschel L H and Piper D R Enzyme Treated Red Blood Cells of Sheep in the Test for Infectious Mononucleosis Am J Clin Path 32 240 1959
28 Nelson R S and Darragh J H Infectious Mononucleosis Hepatitis a Clinicopathologic Study Am J Med 21 26 1956
29 Pader E and Grossman H Thrombocytopenic Purpura in Infectious Mononucleosis New York J Med 56 1905 1956
30 Paul J R and Bunnell W W The Presence of Heterophile Antibodies in Infectious Mononucleosis Am J M Sc 183 90 1932
31 Peterson E T Walford, R L Figueroa W G and Dusholm R Ox Cell Hemolysis in Infectious Mononucleosis and in Other Diseases Am J Med 21 193 1956
32. Pfeiffer E Drusenfieber Jahrb Kinderh 29 257 1889
33 Redmond, A J Infectious Mononucleosis Treated With ACTH New York J Med 54 3411 1954
34 Sadusk, J F The Skin Eruption and False Positive Wasserman in Infectious Mononucleosis (Glandular Fever) Internat Clin 1 239 1941
35 Sawitsky A Papps J P and Wiener L M The Demonstration of Antibody in Acute Hemolytic Anemia Complicating Infectious Mononucleosis Am J Med 8 260 1950
36 Schultz, L E Heterophile Antibody Titer in Diseases Other Than Infectious Mononucleosis Arch Int Med 81 328 1949

37 Shinton N K and Hawkins C F A Fatal Case of Glandular Fever Lancet 271:708 1956
38 Shiver C B Jr and Frenkel E P Palatine Petechiae an Early Sign of Infectious Mononucleosis J A M A 161 592 1956
39 Soloff L A and Zatuchni J Infectious Mononucleosis Associated With Symptoms of Acute Pericarditis J A M A 152 1530 1953
40 Southam C M Goldsmith Y and Burchenal J H Heterophile Antibodies and Antigens in Neoplastic Diseases Cancer 4 1036 1951
41 Sprunt T P and Evans F A Mononuclear Leukocytosis in Relation to Acute Infectious (Infectious Mononucleosis) Bull Johns Hopkins Hosp 31 410 1920
42 Stefanini M and Adelson E Studies on Platelets Heterophile Reaction Due to Forssman Antibody in Patients With Idiopathic and Secondary Thrombocytopenic Purpura, Am J Clin Path. 22 1164 1952
43 Strauss R Infectious Mononucleosis Simulating Acute Appendicitis With Description of a Specific Lesion of the Appendix Am J Clin Path. 12 295 1942
44 Thurm R H and Bassen F Infectious Mononucleosis and Acute Hemolytic Anemia Report of Two Cases and Review of the Literature Blood 10 841 1955
45 Tidy H L and Daniel E C Glandular Fever and Infectious Mononucleosis With an Account of an Epidemic Lancet 2 9 1923
46 Vahlquist B Ekelund H and Tveteras E Infectious Mononucleosis and pseudomononucleosis in Childhood Acta paediat 47:120 1958
47 Wilson S J Ward C E and Gray L W Infectious Lymphadenosis (Mononucleosis) and Hemolytic Anemia in a Negro Blood 4 189 1949
48 Wintrobe M M Clinical Hematology ed 4 Philadelphia 1956 Lea & Febiger
49 Wiseman B K The Treatment of Infectious Mononucleosis J Chron Dis 6 347 1957
50 Beeson Paul B and others (editors) Year Book of Medicine 1957 1958 Serie Chicago 1958 Year Book Publishers Inc

CHAPTER 22

Leukemia—General Aspects and Clinical Features

Leukemia is a disorder of the white blood cells of unknown etiology with a fatal outcome It is generally considered to be a neoplastic change in the blood forming tissues and is characterized by an uncontrolled and disorderly prolifera tion of leukocytes and their precursors with invasive tendencies Although the white blood cells are usually present in excessive numbers in the circulating blood they may be present in normal or reduced numbers In either case immature or abnormal cells are present during a major part of the course of the disease

It is of interest historically that, although cases of leukemia had been pre viously reported by others credit for ascribing the altered state of the blood to an increase in the colorless cells rather than to pus and for naming the condition leukemia (white blood) goes to Virchow [9]

Furth[34] has emphasized the essential identity between leukemia and neoplasms since in both conditions the immature cell has acquired an inability to respond to forces in the host normally regulating their maturation and proliferation Many concepts especially those relating to causation and treatment of leukemias in human beings stem from animal experimentation especially from experiments on leukemia in mice which is similar both pathologically and clinically to that in human beings

No proof exists yet that leukemia in mice is identical with that in human beings although similar effects result in increasing susceptibility following ioniz ing radiation and in parallel therapeutic responses following administration of a variety of chemical agents New mitotic poisons employed in the treatment of human patients have been obtained by screening in mice with experimentally produced leukemia

Classification of Leukemia in Childhood Leukemia is classified as acute subacute or chronic and by the type of white blood cell predominating in the bone marrow and circulating blood No rigid criteria can be applied for designat ing the exact duration of the disease It is much more practical especially since survival has been extended by chemotherapy to employ the terms acute and chronic in relation to clinical severity and to qualify further as to the type of cell or cellular pattern predominating in the disease

37 Shinton N K and Hawkins C F A Fatal Case of Glandular Fever Lancet 271 708 1956
38 Shiver C B Jr and Frenkel E P Palatine Petechiae an Early Sign of Infectious Mononucleosis J A M A 161 592 1956
39 Soloff L A and Zatuchni J Infectious Mononucleosis Associated With Symptoms of Acute Pericarditis J A M A 152 1530 1953
40 Southam C M Goldsmith Y and Burchenal J H Heterophile Antibodies and Antigens in Neoplastic Diseases Cancer 4 1036 1951
41 Sprunt T P and Evans F A Mononuclear Leukocytosis in Relation to Acute Infectious (Infectious Mononucleosis) Bull Johns Hopkins Hosp 31 410 1920
42 Stefanini M and Adelson E Studies on Platelets Heterophile Reaction Due to Forssman Antibody in Patients With Idiopathic and Secondary Thrombocytopenic Purpura, Am J Clin Path 22 1164 1952
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44 Thurm R H and Bassen F Infectious Mononucleosis and Acute Hemolytic Anemia Report of Two Cases and Review of the Literature Blood 10 841 1955
45 Tidy H L and Daniel E C Glandular Fever and Infectious Mononucleosis With an Account of an Epidemic Lancet 2 9 1923
46 Vahlquist B Ekelund H and Tveteras E Infectious Mononucleosis and pseudomononucleosis in Childhood Acta paediat 47 120 1958
47 Wilson S J Ward C E and Gray L W Infectious Lymphadenosis (Mononucleosis) and Hemolytic Anemia in a Negro Blood 4 189 1949
48 Wintrobe M M Clinical Hematology ed 4 Philadelphia 1956 Lea & Febiger
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Acute Stem Cell Leukemia The predominant cell in the bone marrow and peripheral blood in the majority of patients with acute leukemia is usually primitive and there is a virtual absence of cellular differentiation into myeloblastic or lymphoblastic types The morphologic characteristics in these patients do not suggest any specific line of development Although it is possible in rare cases to separate these immature cells the term stem cell leukemia overcomes the difficulties of differentiation The majority of these cells are often classified as lymphoblasts because of their resemblance to lymphocytes in their proximity It is for this reason that acute lymphoblastic leukemia frequently has been regarded as the most common form of leukemia in childhood (For differentiation of cell types occurring in leukemia see Chapter 18) In the more recent reports the terms granulocytic and lymphocytic are used for the leukemias instead of myelogenous and lymphatic

Granulocytic Types Granulocytic types of leukemia encountered in children are of three types acute promyelocytic acute myelocytic and chronic myelocytic or myelogenous

ACUTE PROMYELOCYTIC LEUKEMIA Acute promyelocytic leukemia the most frequent member of the granulocytic group is a disease in which the predominant cells are early myelocytes (myelocytes A and B) The terms acute myeloblastic leukemia and acute promyelocytic leukemia sometimes are used interchangeably in this type because either blast forms or promyelocytes may predominate at one time or another in the same patient However in contrast to the myeloblast which is devoid of granules a few darkly stained granules are found *in the promyelocyte Cases reported as acute granulocytic leukemia* probably fit into this category

ACUTE MYELOCYTIC LEUKEMIA In patients with acute myelocytic leukemia the blood smear and especially the bone marrow are characterized by the presence of more mature granules than those of the promyelocytic form The cells possess numerous granules scattered throughout the somewhat grayish or basophilic cytoplasm Because of the greater number of granules and the more advanced maturity this type of cell is often termed myelocyte C in contrast to less mature *myelocytes A and B containing fewer granules characterizing the promyelocytic* form Acute myelocytic leukemia is differentiated from the chronic myelogenous type not only by the more moderate leukocytosis but also by the narrow range of maturity of the abnormal forms One of the features of acute myelocytic leukemia is the cytologic resemblance in many of the cells to the monocyte especially with regard to the nucleus.

CHRONIC MYELOCYTIC (GRANULOCYTIC MYELOID, OR MYELOGENOUS) LEUKEMIA Chronic leukemia is infrequent in childhood being confined to the chronic myelocytic type which accounts for about 2 per cent of all leukemias in this age period Hyperleukocytosis is common with segmented and nonsegmented neutrophils constituting 75 per cent of the total Myelocytes and metamyelocytes are present in increased numbers The bone marrow may show larger numbers of myelocytes than the peripheral blood although samples from both areas are characteristically similar Relatively few myeloblasts or immature myelocytes are found at any time except terminally Basophils are increased and platelet counts

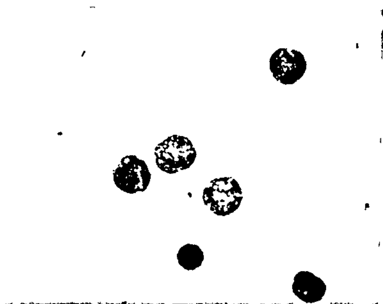


Fig 35 Photomicrograph from blood smear of a patient with acute leukemia Note lighter staining and finely granular or stippled appearance of nucleus in lymphoblasts in upper section Compare with dark staining basichromatin of nuclei of two normal lymphocytes in lower section (×1200)



Fig 36 Photomicrograph of bone marrow smear from a patient with acute leukemia (×1200) Note uniform infiltration by lymphoblast

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are either normal in number or may reach levels of 1 million per cubic millimeter or more In patients with chronic myelogenous leukemia the neutrophils contain very little alkaline phosphatase, whereas in those with pyogenic infections associated with a leukocytosis or a leukemoid reaction corresponding cells exhibit a great increase in this enzyme [4, 94a, 9, 101] Auer bodies rod shaped structures in the cytoplasm which are peroxidase positive may be present in the myeloblasts or myelocytes As the disease progresses anemia and thrombocytopenia prevail as in patients with the acute leukemias

The disease is rare in childhood but is often seen in the first months of life as a form of congenital leukemia In affected infants the cells are as well differentiated as they are in patients with the adult form of the disease but in contrast to the prolonged survival in the latter the course in the infant is short The older child may survive for a period of several years Nevertheless the designation chronic myelocytic leukemia in the young patient refers to the type of blood picture rather than to the length of the survival

The onset of the disease is particularly difficult to designate in patients with chronic myelocytic leukemia because the symptoms are vague A routine blood count made because of continued low grade fever and fatigue frequently calls attention to an underlying hematologic disorder The marked leukocytosis with a shift of the neutrophils to the left and the presence of a few metamyelocytes and myelocytes suggest a diagnosis of a leukemoid reaction initially Hepatosplenomegaly and lymphadenopathy are usually marked in patients with this form of leukemia At the onset these features may be absent except for a palpable spleen which enlarges as the disease progresses Pain about the hip may become so pronounced that walking is painful The marked limp which develops early rarely correlates with an abnormal finding in the roentgenogram

Monocytic Leukemia Monocytic leukemia is similar to the other types in most respects The onset may be insidious with a fatal outcome in a few weeks or months or it may be sudden with the disease running its course within a few days In patients with the latter type of onset the spleen is slightly enlarged and the liver and lymph nodes may not be palpable The disease has an age incidence ranging from early infancy to later childhood The symptoms and physical signs are similar to those of other types of leukemia except that hypertrophied and spongy gums have been described more frequently in patients with the monocytic type

Two types of monocytic leukemia are described When the blood picture is marked by the simultaneous presence of both myelocytes and monocytes or immature cells intermediate between myeloblasts or myelocytes and monocytes the disease is designated as the Naegeli type or myelomonocytic leukemia When the immature cells resemble monocytes and reticuloendothelial cells the disease is designated as the Schilling type (See Chapter 18)

Chronic monocytic leukemia is extremely rare in childhood Only two patients in this category were observed in a series of 900 cases of childhood leukemia [1] The disease may be difficult to diagnose in the early stage because of predominant megaloblastic blood and bone marrow changes diverse skin lesions and spontaneous remissions However the finding of varying numbers of cells resembling

adult monocytes and their precursors in the peripheral blood and bone marrow should arouse suspicion of the underlying disease Early in the course of the disease there may be a marked leukocytosis or a leukopenia

Eosinophilic Leukemia Eosinophilic leukemia is an acute disease of childhood in which the majority of cells in the peripheral blood and tissues are mature eosinophils but in some cases are associated with myeloblasts and myelocytes Enlargement of the liver spleen and lymph nodes anemia and thrombocytopenia may not be present until late in the disease The blood picture may change to that of an acute myeloblastic leukemia but more often tissue infiltration and the peripheral blood disclose invasion by the adult eosinophil The slight elevation in the number of myeloblasts occasionally encountered is not sufficient for the diagnosis of acute leukemia [18 3 39]

In a 9 year old boy with eosinophilic leukemia the leukocytes which numbered 88 000 per cubic millimeter of blood (80 per cent mature eosinophils) were associated with diffuse parenchymal infiltration of both lungs and intermittent fever In this patient steroids (prednisone) produced a temporary regression of lung infiltration and symptomatic relief Postmortem examination revealed widespread tissue infiltration with mature eosinophils

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In terms of clinical severity stem cell promyelocytic monocytic chronic myelocytic and eosinophilic leukemias are acute leukemias in childhood Chemotherapy and other modern supportive measures however have modified their course and prolonged life It should be borne in mind that acute stem cell leukemia is essentially a disease of infancy and childhood although no age group is spared In rare instances chronic myelocytic leukemia in children will run a prolonged course simulating that in the adult Aleukemic leukemia is the designation for leukemia in patients in whom there is either no leukocytosis or a marked reduction in leukocytes but usually with the presence of abnormal cells in the peripheral blood and tissue histology showing the true nature of the disease Chronic lymphocytic leukemia is the most protracted and benign type of leukemia and occurs invariably in adults

Incidence Age Distribution Sex and Frequency of Types Acute leukemia is commonest between 2 and 5 years of age with the incidence rising from a moderate elevation in the first two years of life to a peak in the third and fourth years [1] Although acute leukemia predominates in the first four to five years of life myelocytic leukemia is the commonest form of the chronic disease up to the age of 60 years after which the lymphocytic type is most frequently encountered In total numbers chronic myelocytic leukemia and chronic lymphocytic leukemia are more frequent in the groups over 50 years of age [4 37 9]

According to Cooke [1] there is a gradual increase in the proportion of males to females who have acute leukemia with the predominance of boys being greater in later childhood than during infancy In the first year of life more cases are observed in girls than in boys with this exception leukemia is more commonly observed in the male than in the female at all other ages

Mortality statistics show that there has been a progressive increase in the death rates from leukemia In patients in the youngest age group (0 to 4 years) the rate

has risen from 2 2 to 5 1 per 100 000 deaths between 1930 and 1949 [4] In one study[78] the incidence in one large hospital from 1932 to 1948 was 0 42 per cent of all admissions to the general pediatric service Figures showing the changing incidence of leukemia cannot be truly ascertained from hospital admissions alone since there is a tendency for patients with leukemia, especially in the younger age groups to be concentrated in large hospitals and medical centers However the recorded death rate from leukemia at all ages has risen steadily in the United States from 1 4 in 1921 to 6 3 in 1953[5] An over all mortality rate from leukemia of approximately 10 per 100 000 has been estimated for the United States[94b] In recent years a similar increase in all types of leukemia has also been noted in England and Wales In Scotland an approximate doubling of the incidence of admissions for leukemia to several large hospitals was recorded during the period from 1938 to 1951[35]

Within six months we have observed three unrelated children under the age of 5 years with acute leukemia living within one half mile of each other Intensive investigation however revealed no known leukemogenic agent to which the three children had been exposed This relationship in so many aspects seemed a startling experience and may eventually prove to be more than coincidental

Etiology Various physical chemical and metabolic agents have been found capable of precipitating or activating the development of leukemia Infectious agents trauma[103] and exposure to drugs and chemicals have appeared to be so closely associated with leukemia as to suggest a causative relationship Arsenicals sulfonamides and benzol poisoning[60] have been prominently mentioned but evidence is still incomplete The widespread use of antimicrobial and antibiotic agents in the treatment of patients with infectious disease has led to the impression that they may possess leukemogenic activity although there has been no proof of this relationship

The morphologic analogy between the maturation arrest in the bone marrow of patients with pernicious anemia and the blast cells in those with acute leukemia has suggested that leukemia represents a metabolic or deficiency disease but treatment on this basis has given negative results[19] In another theory leukemia is based on disordered hormonal or metabolic functions In one group of experiments[84] specific substances that produce myeloid and lymphoid proliferation and maturation in animals have been extracted from the urine of patients with leukemia Data have also been obtained supporting the concept that the failure of cell maturation in patients with leukemia is associated with a loss of factors which inhibit cell proliferation [4] Leukemia has also been compared to a type of autoimmunization involving the proliferation of one of the white cell forming tissues in which the response once begun is self replicating and permanent[30a] The stimuli for such leukemogenic activities are ionizing radiation carcinogenic chemicals and viruses

Infections The role of infections in the causation of leukemia receives periodic emphasis Cooke [1] has pointed out that infection frequently antedates the development of acute leukemia in childhood and that the character of the age incidence for leukemia tends to parallel the frequency of acute infections One of the difficulties in accepting the infectious concept of leukemia however has

been the well-established observation that in human beings with leukemia, as in experimental animals with leukemia there is the lack of communicability which characterizes other infectious diseases

Viral Etiology With the possibility that nonspecific infections conceivably may be causally related to acute leukemia of childhood a number of studies have focussed their attention on a viral etiology for human leukemia The demonstration by Ellerman and Bang[31] that chicken lymphomatosis could be transmitted by filtered extracts stimulated interest in the causation of leukemia and allied diseases in other species as well as in human beings Gross[40] demonstrated a viral etiology for lymphoid leukemias in mice which is noncontagious but is communicated to offspring during embryonal life through either the male or the female parent resulting in leukemia after a period of latency of one third to one half of the life span of the animal According to this hypothesis leukemia develops when the dormant agent is activated any time during the life span of the mouse The viral agent thus exists in inactive form manifesting malignant capabilities only when challenged by as yet obscure but presumably trigger stimuli such as x rays, chemicals infections or products of endogenous metabolic activities Also the virus would not necessarily become activated during the life span of a single carrier host but would be transmitted from one generation to another in a vertical pattern

Schwartz and Schoolman[81] extended these observations to show that cell free filtrates prepared from the brain of patients who died from acute leukemia are capable of accelerating the development of or inducing this disease in a leukemic strain (AKR) of mice These studies based on animal experimentation are provocative and provide a basis for explaining many of the features of human leukemia seen in clinical practice The confirmation for this hypothesis in human leukemia however awaits the discovery of the virus in man A detailed comparison between any type of human leukemia with the response of an inbred animal strain to any of the leukemogenic virus has still to be justified [91b]

Heredity The agents found to be leukemogenic or to accelerate the onset of leukemia in mice depend upon the genetic constitution of the mouse [43] In the human being as well attention has been drawn to the possibility that the genetic constitution may condition susceptibility to leukemia Many instances have been reported in which leukemia developed simultaneously in several members of the same family or in successive generations The most striking family history is that reported by Anderson[3] in which five out of eight siblings developed leukemia at 5 to 8 years of age The occurrence of leukemia in twin children especially in identical twins has been the subject of several reports [4, 41] Gunz and Dameshek[41] reported a family in which a man aged 53 years died of chronic lymphocytic leukemia twenty seven years after his father and the father s identical twin died of the same disease both at the age of 56 years

Analysis of large series of cases in any one hospital provides little evidence however that heredity is of any importance in human leukemia On the other hand in the light of experimental and unequivocal evidence of multiple cases in one family the hereditary factor cannot be categorically dismissed Videbaek [96] for instance finding a familial incidence of leukemia of about 8 per cent in

Denmark as compared with 0 5 per cent in control families concluded nevertheless that leukemia was a matter of an inherited predisposition rather than of direct chromosomal inheritance

Leukemia has never been known to pass the placental barrier either in the mouse[16] or in the human being In the experimentally produced disease the leukemic cells are found only on the maternal side of the placenta the fetal circulation remaining normal Experience has been universal that leukemic mothers give birth to normal infants the placenta apparently serving as an effective barrier [10]

A probable exception to this observation is the recent report of a mother with signs and symptoms of leukemia beginning in the seventh month of pregnancy in whom acute lymphocytic leukemia was diagnosed eight days postpartum and whose infant developed the same disease at 9 months of age [6] *The problem here is one of establishing an absolute relationship between leukemia in the mother and infant because of the lapse of time until the disease was* noted If transplacental passage of the agent occurred late in pregnancy it is theoretically possible for the disease not to have become manifest until some time after birth [6]

Radiation In addition to the long term genetic effects of ionizing radiation [47 48] there is mounting evidence that this agent is strongly implicated in the causation of leukemia and is a major factor in the steadily increasing death rate from this disease The evidence for irradiation as a leukemogenic agent in human beings includes the increased incidence of leukemia among radiologists as compared with other physicians [61] in patients receiving radiation therapy for ankylosing spondylitis [1] in children who received radiation to the mediastinum for enlarged thymus in infancy [56 8] and among atomic bomb survivors in Hiroshima and Nagasaki [33 6 68] Hematologic surveys in Hiroshima indicated that after atomic radiation there was a "latent period" of several years before leukemia was in evidence Chronic myelogenous leukemia was most common followed in frequency by acute myelogenous and acute lymphatic types [66]

It has been postulated that the increased exposure of the population to ionizing radiations employed in medicine and dentistry also contributes to the accelerated death rates from leukemia [9] *There is a possibility that the spontaneous incidence* of leukemia may be due to radiation from natural background sources Quantitative estimates have been made of the probability of developing radiation induced leukemia in the average person in a population [8]

It is of interest that age has been shown to be a factor of importance in experimentally induced leukemia Animals exposed to whole body irradiation before puberty develop a far higher incidence than older animals [0] Besides causing genetic damage diagnostic pelvimetry during pregnancy or threatened abortion may occasionally cause leukemia or cancer in the unborn child [9]

In the light of these concepts it would appear that certain genetic influences acting in conjunction with both identified and as yet unidentified external factors transform normal hematopoietic cells and their precursors into those with an unrestrained and purposeless growth [93] Exposure to ionizing radiation in the environment infections contamination of the atmosphere with chemical pol

lutants and marrow depressings drugs and chemicals may serve as such activating agents[60] Despite these incriminating data for the carcinogenic effect of radiation it is not possible yet in man to determine whether or not a threshold dose for the induction of leukemia does or does not exist [6a]

Preleukemic Stage of Leukemia Cases of leukemia have been recorded in which a nonleukemic blood disorder of variable duration preceded the onset of acute leukemia[3] This preliminary period is usually characterized by a deficiency of one or more cellular elements in the peripheral blood resulting in anemia neutropenia thrombocytopenia or combinations thereof The bone marrow is moderately hyperplastic and may or may not show an arrest in the granulocytic series[13] In one patient the preleukemic stage extended over a period of nine years and was punctuated by a remission before the terminal development of myeloblastic leukemia[100]

The anemia is frequently hemolytic in nature although not always accompanied by an elevated reticulocyte count.[6] In one case in our experience acute stem cell leukemia in a child 4 years old was preceded two years earlier by severe iron deficiency anemia Neither the blood nor bone marrow showed evidence of immature white blood cell precursors In the intervening period the child was asymptomatic without changes in the blood status In children prolonged neutropenia and leukopenia or aplastic anemia may appear to precede acute leukemia but when this sequence is evaluated further it appears possible that they represent a remission phase in the course of the latter disease Abnormal cells indicative of leukemia are not found in the blood or bone marrow of patients with these antecedent disorders and there is no associated splenomegaly or lymphadenopathy Still to be determined by a more direct approach than circumstantial evidence is the extent to which the initial disorder is involved in the subsequent development of leukemia

Leukemia in the Newborn Period (Congenital Leukemia) Congenital leukemia is a rare disease of which only fortv five adequately documented cases have been reported [4] Some infants exhibit signs of leukemia at birth and succumb shortly thereafter In another group the infant appears normal at birth but develops clinical and hematologic signs later in the newborn period In the third group the disease is not detected until the third to the sixth week of life[13a] with a suggestive history of a hematologic disturbance dating back to the first weeks of life

The leukemia is principally myelogenous and is marked by a hyperleukocytosis and a predominance of promyelocytes and myelocytes Myeloblasts vary from 10 to 80 per cent The granulocytic leukemia is noted both in the blood and bone marrow[9, 7, 1, 275] Anemia is uncommon in the newborn infant but develops soon thereafter with a rapid progression to pancvtopenia Large numbers of nucleated red blood cells may be present in the peripheral blood regardless of anemia Those cases that develop later show anemia when diagnosed Platelets are reduced in number Physical signs include hemorrhages of the skin bleeding from the mucous membranes and umbilical stump nodular skin infiltration and enlargement of the liver and spleen

In the newborn period leukemia is to be differentiated principally from the

leukemoid reaction of sepsis and congenital syphilis erythroblastosis and congenital thrombocytopenic purpura In patients with a leukemoid reaction myelocytes as well as segmented and nonsegmented polynuclear cells are increased but myeloblasts are absent Eventually the causative agent of the sepsis is discovered by blood culture or by evidences of specific organ involvement with infection The essential differentiating feature is the extensive infiltration of the nonhematopoietic organs as well as the liver spleen and lymph nodes by immature cells of the granulocytic series in infants with leukemia and the absence of such infiltration in patients with a leukemoid reaction Erythroblastosis which may also be accompanied by a leukocytosis anemia, and myelocytes in the peripheral blood can be diagnosed by evidences of blood group incompatibility Although the decreased number of platelets in patients with congenital thrombocytopenia may have to be considered hepatosplenomegaly is absent and promyelocytes and myelocytes do not appear in the blood smear

It is of interest that no case of congenital leukemia has ever been encountered in the newborn infant whose mother had leukemia during pregnancy Furthermore leukemia diagnosed soon after birth must have had an intrauterine origin without affecting the mother

Treatment of leukemia with current methods (see Chapter 23) is ineffective and the duration of life varies from several days to two or three months This rapidly fatal course is in contrast to the prolonged course of chronic myelogenous leukemia in adults and in children beyond infancy [3]

The presence of associated cardiac orthopedic skeletal and other developmental anomalies in patients with congenital leukemia[9] is analogous to the well documented combination of leukemia and mongolism [6 63 8] The concept which attributes mongolism to toxic factors of endogenous or exogenous origin inducing changes in the fetus during the sixth to the ninth week of pregnancy[46] may apply to both sets of circumstances with simultaneous and parallel injury to the developing bone marrow In each instance the occurrence of a predominately myeloid form of leukemia suggests that the granulocytic precursors rather than the lymphoblasts were principally affected

Spontaneous Remissions Complete and spontaneous clinical and hematologic *remissions have been observed in some patients without apparent relation to* therapy They have also occurred in patients on supportive therapy including transfusions or without antibiotics and especially following infections An incidence of spontaneous remissions as high as 10 per cent of cases has been given in one series in children[30] and as low as only 1 in 105 children with transfusions as the sole therapy [30] Remissions following infections have been described more frequently Remarkable improvement and complete and dramatic arrest of the disease have been reported in a group of children with acute leukemia following infection caused by streptococcus staphylococcus and varicella and by inoculation with the feline panleukopenia virus [11] Four spontaneous remissions were observed *in a young child over a seventeen month period on supportive therapy* and antibiotics Characteristic of the majority of cases was an antecedent stage of severe leukopenia frequently a leukopenic agranulocytic phase and hypoplasia or aplasia of the marrow associated with anemia It is of interest that thera

peutically induced remissions also demonstrate a hypoplastic phase preceding remission This transformation to a normal status is usually short lived varying from several weeks to a few months and in one adult reported on complete remission lasted twenty one months[1] Following the remission however the disease returns to its original severity uninfluenced by the temporary reversal to normal

Clinical Features The clinical features of leukemia combine the constitutional manifestations of the disease blood deficiencies particularly thrombocytopenia and anemia and involvement of organ systems

Symptomatology The clinical manifestations and course of acute leukemia are strikingly similar and show no differences with respect to the predominating cell type The onset of this disease is insidious with localized symptoms obscuring the systemic nature of the disease Symptomatology often follows a well defined pattern and several sets of symptoms are observed Frequently the onset is ill defined with the patient exhibiting pallor anorexia irritability abdominal pain and malaise These symptoms may pass unnoticed until diagnosis by blood examination prompts a retrospective review of the history Other patients have persistent low grade fever with intermittent periods of moderate or marked temperature elevations which either are unexplained or are attributed to a sore throat In some the disease is ushered in by high fever and an upper respiratory infection from which the child does not recover as expected with routine treatment In still others there is the appearance of hemorrhagic manifestations such as bleeding from the gums epistaxis and a tendency to bruise Persistent hemorrhage after a minor operation or after tonsillectomy may be the first evidence of acute leukemia

More rarely the onset is abrupt with the clinical picture of an acute infectious disease marked by hyperpyrexia severe prostration nausea vomiting abdominal pain anemia and purpura In a patient with this type of fulminating disease with a duration of several days the spleen and lymph nodes may be only slightly or moderately enlarged Symptoms such as local or generalized pain limp and bruising dating from a specific injury are sometimes so definitive as to justify a consideration of trauma as an etiologic factor Migratory bone and joint pains and at times acute arthritis with signs of local inflammation pallor weakness epistaxis and an irregular febrile reaction simulate the clinical picture of rheumatic fever Spontaneous rupture of the spleen is a rare occurrence in patients with acute leukemia and may produce the initial symptomatology of the disease[10]

Physical Findings Early in the course of acute leukemia the physical examination may be essentially normal Sooner or later the lymph nodes usually enlarge and the edge of the spleen is palpable on deep inspiration Lymph node enlargement is most marked in the cervical region but may be conspicuous over the occipital and postauricular areas and elsewhere over the scalp It is often less pronounced than in patients with chronic leukemia Involvement of the lymphoid tissue resulting in bilateral painless enlargement of the salivary and lacrimal glands is designated as the Mikulicz syndrome[11] The liver and spleen may be enormously enlarged but the spleen may fluctuate in size especially during treatment with little accompanying change in the liver

Hemorrhages into the skin consist of petechiae purpura and extensive ecchy

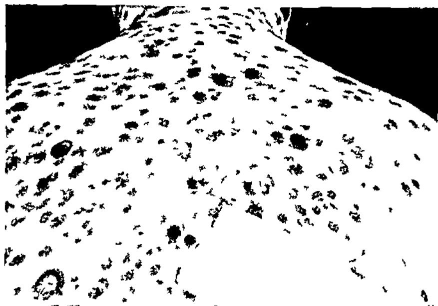


Fig. 37 Skin lesions in child with acute lymphoblastic leukemia (leukemia cutis) The lesions originally discrete were later confluent and covered the face trunk and eventually the entire body The individual lesions were slightly nodular erythematous and purpuric Many were eventually necrotic

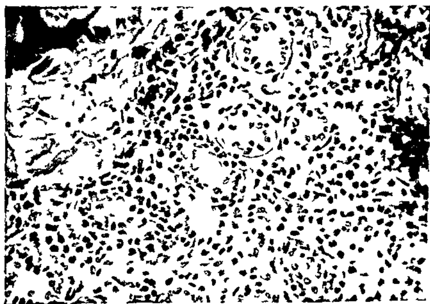


Fig 38 Biopsy of skin lesion from patient shown in Fig 37 Note leukemic cells densely infiltrating corium about sweat glands (×435)

moses which recede and reappear during the disease Hemorrhages into eye grounds and in mucous membranes especially in the mouth and nose are common Ulcerations in the buccal mucosa result from leukemic infiltration as well as from hemorrhage

Swollen spongy hypertrophied gums to the extent that the teeth are hidden which characterizes monocytic leukemia *may also be found in patients with* acute stem cell leukemia Ulcerations may extend from the gums to the tonsils and palate

Skin involvement is varied It is either specific with leukemic infiltration or nonspecific with erythema multiforme like papuloneurotic and eczematous lesions *More specific are the small yellow discrete* and elevated flat topped plaques which are either widespread or confined to localized areas on the abdomen and extremities Infrequently maculopapular eruptions cover the face initially and spread to the chest and back and eventually to the abdomen and extremities Each lesion varies from 1 cm or more in diameter may become hemorrhagic and necrotic and is eventually covered by a grayish membrane Biopsy reveals extensive leukemic infiltration around blood vessels and adnexa in the epidermis *and subcutaneous tissue*

Mediastinal involvement should be suspected in the presence of symptoms of respiratory distress The kidneys are often greatly enlarged and glomerulone phritis may precede or accompany the overt blood changes of leukemia

Skeletal Changes The encroachment upon actively functioning marrow by leukoblastic cells within the bony skeleton at a period when nonfunctioning yellow marrow is not yet available accounts for the changes observed by roentgenography *in patients with this disease Skeletal lesions and symptoms are less common in* adults since they possess large amounts of fatty marrow which can be replaced by leukemic cells before osseous changes occur[2 49 88]

Bone changes consist of transverse bands of diminished density osteolysis and periosteal elevation with new bone formation Most commonly multiple areas of destruction and production are seen at variable skeletal sites The evidence of skeletal lesions varies in different series being given as 50 per cent in one group[5] and 73 per cent in another [8] *Bone pain develops with increased intraosseous* tension secondary to the proliferation of the leukemic cells Less frequently bone pain is caused by infiltration of the synovial membranes of several joints by leukemic cells These eventually destroy the spongiosa producing local or diffuse rarefaction Leukemic cells penetrate the cortex of bone and extending beyond it lift up the periosteum which becomes *the site of new bone formation* The lifting of the periosteum also results from subperiosteal hemorrhages secondary to throm bocytopenic purpura

An important diagnostic feature is the marrow translucent zone just proximal *to the metaphysis of the shaft of the long bone*[a] which is most marked in areas of most rapid growth the distal femur tibia radius and ulna in decreasing order of frequency These zones of diminished density are best demonstrated in the tibia and femur at the knees Osteosclerosis occurs least frequently and may be either focal or diffuse and is secondary to excessive formation of spongiosa

The changes observed by roentgenographic examination may be summarized as follows (1) osteoporosis (2) transverse lines of increased density (growth arcas) (3) radiotranslucent bands[6] (metaphyseal and epiphyseal) (4) osteolytic lesions (cortical and medullary) (5) cortical thinning and destruction (6) leukemic infiltrations and hemorrhage and (7) periosteal (lifting of periosteum new bone formation)



Fig 39



Fig 40

Fig 39 Skeletal lesions of leukemia Note generalized and local rarefaction and focal areas of bone absorption associated with replacement of bone marrow by leukemic tissue

Fig 40 Arrow points to a narrow deep transverse zone of rarefaction just proximal to the metaphysis of the long bones usually most marked in the lower ends of the femurs and tibias Note also multiple areas of rarefaction in medullary portion of bones

Course The course of acute leukemia is beset by many complications despite the fact that with chemotherapeutic agents and supportive therapy the patient is free of symptoms for prolonged periods and temporary remissions are induced The major problems arise from a tendency toward hemorrhage local or systemic infection and leukemic infiltration of tissues and organs resulting in disturbances from their increased size and interference with function

Hemorrhage The hemorrhagic manifestations of leukemia can usually be ac

counted for by a reduction in the number of platelets although bleeding need not necessarily occur with it Why one patient bleeds while another with comparable thrombocytopenia does not is puzzling The depletion of platelets has been attributed to the depression of megakaryocytes by replacement of bone marrow by primitive cells Hypoprothrombinemia and factor V deficiency due to liver damage have been demonstrated in some patients but there is no consistent relationship with any of these factors Circulating fibrinolysin resulting in a fibrinogenopenia with massive hemorrhage has also been reported

The control of bleeding by ACTH and the adrenal steroid hormones without resulting increase in the number of platelets suggests however that vascular dysfunction may result from causes other than thrombocytopenia

Besides skin involvement with petechiae and ecchymoses serious bleeding can occur in more strategic areas Intracranial hemorrhage is a frequent terminal complication especially when the number of circulating abnormal cells is high For this reason the additional hazard of the hypertensive side effects of ACTH and steroids must be watched for during treatment Epistaxis hematuria gastrointestinal hemorrhage and oozing from the gums ulcerations of the buccal mucous membranes and interruptions in the mucocutaneous junctions occur frequently especially in the later stages of the disease Episodes of severe bleeding occur repeatedly with extreme degrees of thrombocytopenia in both phases of agranulocytosis and leukemic leukocytosis when the bone marrow is heavily infiltrated Chemotherapeutic agents such as amethopterin (Methotrexate) administered for prolonged periods or in excessive amounts may produce toxic effects such as ulcerations and bleeding in the buccal mucosa and lips and in the gastrointestinal canal Necrotic lesions are often present in sites of ulcerated and hemorrhagic areas especially about the mouth

Anemia Diagnostically the red cells are both normochromic and normocytic and anisocytosis poikilocytosis and achromia are minimal Occasionally large numbers of elliptical and oval cells are present The crowding out of the normal bone marrow elements and their precursors by leukemic tissue has been offered as a cause of thrombocytopenia anemia and agranulocytosis The mechanism of the anemia cannot be uniformly correlated however with the extent of bleeding or erythropoietic failure Also the appearance of the bone marrow with regard to the extent of leukemic invasion is often unrelated to the degree of anemia

The pathogenesis of the anemia is now viewed more as a dynamic process than a crowding out of erythroid elements [84] It has been demonstrated by cell tagging techniques [14, 8] that the anemia of chronic leukemia is due in part to a decreased survival of erythrocytes perhaps caused by an intrinsic corpuscular defect A similar increased hemolysis is also an important factor in advanced nonlymphomatous neoplastic disease [4] When red cell production and red cell destruction were measured simultaneously in patients with leukemia no single mechanism could be established for the causation of the anemia in every case There was extreme variability between the degree of red cell survival (normal or short) and adequate or inadequate compensatory red cell production Multiple factors therefore contribute to the anemia

Pallor fatigability dyspnea on exertion and listlessness are attributable to

the anemia The increased basal metabolic rate in patients with acute leukemia is responsible for these symptoms as well as for anorexia weight loss and irregular fever

Relationship of Hyperleukocytosis to Severity of the Disease The patients who present themselves with a marked leukocytosis at the onset are much more severely affected and the disease may run a more fulminant course than in those with leukopenia When the disease has become stabilized whether spontaneously or by therapy and the cells are reduced in numbers the clinical course is not significantly different from that of patients with a moderate leukocytosis or a leukopenia

Infections and Fever The increased susceptibility of the patient to infection is attributable to the absence of granulocytes in the peripheral blood It has been shown also that in persons with acute leukemia phagocytosis of Brucella organisms by mature neutrophils was not impaired[83] and that the absolute mature neutrophil count when infection develops varies from patient to patient It is remarkable however that children can go for long periods with an agranulocytic blood picture free from infection without the continuous use of antibiotics Other factors therefore must account for the development of bacterial infections in patients with acute leukemia The bouts of fever often with hyperpyrexia usually are ascribed to infection but the source is not always discernible The predominance of negative blood cultures in our experience is probably the result of the immediate use of antibiotic and antimicrobial therapy with the onset of fever Hemolytic *Staphylococcus aureus Escherichia coli* streptococci and monilial infections have been the most frequent offenders in our series In other reported series *Bacillus proteus* is also included [3]

Infection contributes to the hemorrhagic tendency and its control often coincides with subsidence of the bleeding Localized infection in soft tissues leads to necrotic lesions requiring persistent and assiduous treatment but frequently remaining unhealed Although usually confined to the oral cavity monilial infections are particularly troublesome and may become widespread In one of our patients moniliasis extended to the blood vessels of kidneys and spleen producing extensive infarcts Despite conflicting experimental data there is suggestive evidence that the increased incidence of infection and tendency for this complication from ordinarily minor lesions and from organisms of low virulence seen in patients receiving the steroid hormones may be due to a failure of the tissues involved to localize the infection in a normal fashion [6]

In a recent series[84] of ninety two febrile episodes encountered in patients with acute leukemia *fifty nine appeared to be related to infection In the remaining* thirty three episodes bacterial viral or fungal infections could not be incriminated as a cause for fever In this group the most common clinical illnesses associated with the proved infections were pharyngitis pyelonephritis and septicemia The most frequently encountered organisms were *Escherichia coli Micrococcus pyogenes* var *aureus* coagulase positive and *Pseudomonas aeruginosa* The cause for persistent fever in the patients who had no demonstrable infection remained obscure Penicillin streptomycin and tetracycline were the common drugs employed

Gastrointestinal Disturbances The vomiting of blood may be caused by persistent oozing from the nasopharyngeal area or from ulcerations in the esophagus and stomach Severe abdominal pain constitutes one of the most distressing symptoms and the source may not be obvious even on postmortem examination The small punctate hemorrhages in the gastric mucosa submucosal hemorrhage enlarged Peyer s patches infiltration of the mesentery of the small bowel and marked enlargement of the abdominal lymph nodes could account for the abdominal pain *For children under treatment with ACTH and steroid hormones* gastrointestinal ulceration and perforation are rarely found by the roentgenography or examination of the tissues but these should not be overlooked Necrotic ulcerative lesions of the lower rectum are associated with severe and persistent agranulocytosis as well as with monilial infection

Specific Organ Changes The enlargement of superficial lymph nodes may be marked or they may be of normal size

The liver and spleen are very moderately enlarged and only occasionally reach enormous proportions usually toward the end of the disease The size of the spleen fluctuates spontaneously or with treatment the liver remains more resistant to change In patients with chronic myeloid leukemia the spleen shows progressive enlargement

Rarely, the lacrimal and salivary glands in children may be involved in a symmetrical painless enlargement designated as the Mikulicz syndrome[44]

Pleural effusion or pneumonia or both may be prominent during the disease In one patient with eosinophilic leukemia confirmed by postmortem examination persistent cough and fever were associated with extensive infiltration of both sides of the chest

The lungs[69a] and kidneys[0] are most frequently involved but the degree of infiltration of these and other organs is not necessarily correlated with the extent of their dysfunction The kidneys frequently are markedly enlarged by the extensive parenchymal infiltration which is difficult to eradicate Gross hematuria is common but in some patients it is confined to the terminal phase whereas in others it appears at the onset Uremia is usually associated with very large kidneys The increase in blood and urinary excretion of uric acid in patients with leukemia[77] results from the disintegration of large numbers of primitive cells *with excessive catabolism of nuclear protein It has been suggested that this* factor coupled with the effects of infiltration may seriously interfere with kidney function It has been stressed that the use of potent chemotherapeutic agents is capable of producing rapid and extensive destruction of blast cells with injurious effects on the kidney The impairment of kidney function however is not a universal occurrence

In a study conducted in a group of thirty five children with leukemia it was concluded that there was no evident impairment of renal function *during the* course of leukemia despite the presence of gross and microscopic infiltration of the kidney parenchyma at postmortem examination[80] On the other hand it has been stressed that impairment of renal function with delayed excretion markedly increases the blood level and antileukemic drugs such as amethopterin produce severe toxicity[17] *These discordant opinions emphasize the need for individualiz*

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ing cases of acute leukemia in children especially with respect to the susceptibility to drug administration

The testes are infrequently involved in patients with acute leukemia When affected they show progressive enlargement with nodular formation due to localized infiltration with leukemic cells

The central nervous system is frequently involved in its many component structures by hemorrhage thrombosis and infiltration by leukemic cells[6] Neurologic signs and symptoms were present in 20 to 25 per cent of the patients in one series Hemorrhage into the brain sufficiently extensive to be the immediate cause of death occurred in approximately 29 per cent[98] Subarachnoid hemorrhage is also to be included in nervous system complications

Exophthalmos due to retrobulbar hemorrhage or infiltration of the bones about the orbit is to be differentiated from chloroma which it closely resembles Retinal hemorrhages are common and occasionally extensive and may be associated with impaired vision or actual blindness Although these hemorrhages are ordinarily a valuable sign of intracranial bleeding they may be found in leukemic patients without other signs of nervous system involvement[98]

The cranial nerves especially the sixth and seventh may be infiltrated and involvement is diagnosed by specific neurologic signs and symptoms[9]

Increased intracranial pressure is a serious prognostic sign and is due to leukemic infiltration primarily of the meninges and to a lesser extent of the cerebral parenchyma It may be asymptomatic but is frequently accompanied by severe headache vomiting meningismus papilledema drowsiness and coma The cerebrospinal fluid is clear under increased pressure the protein and cell count are elevated and microscopic examination reveals blast forms and a low or normal concentration of sugar Roentgenograms of the skulls of the younger children show separation of the sutures It has been suggested[94] that there has been a greater incidence of this complication in patients treated with the hormones and chemotherapy than in previously untreated patients According to this premise the brain is attacked later than are the other organs so that prolonged survival permits the eventual involvement of the central nervous system There is the possibility also that such agents as 6 mercaptopurine[4] and amethopterin cross the blood brain barrier in greatly reduced amounts even though the disease is under control elsewhere The latter feature should be emphasized namely that intracranial complications frequently occur while the peripheral blood and bone marrow are in a complete hematologic remission

Leukemic meningitis occasionally simulates the bacterial form with cloudy fluid and decreased sugar content but examination of the cells reveals its leukemic etiology[4]

Differential Diagnosis Usually leukemia in childhood is diagnosed without difficulty after examination of the peripheral blood and bone marrow In equivocal cases leukemia must be differentiated from disorders in which overlapping occurs in some aspect of the hematologic picture The most important feature of the diagnosis of childhood leukemia is the critical evaluation of the clinical picture so that leukemia is suspected and the peripheral blood and bone marrow are examined

Normal Leukocytic Changes in Infancy and Childhood Normal leukocytic changes in infancy and childhood may simulate leukemia The lymphocytic increase in the first years of childhood must be kept in mind so that unnecessary significance is not attached to conditions in which these cells predominate A mistaken diagnosis of leukemia can be made in young children because of a moderate elevation in the number of white blood cells a preponderance of lymphocytes the presence of small or moderately sized cervical nodes and a palpable spleen In these children the white count is at the upper limit of normal as is the lymphocyte count Furthermore the finding of enlarged cervical lymph nodes and careful search of many smears fail to reveal blast cells The cervical nodes are enlarged from repeated tonsillar infections and a palpable spleen with a smooth edge is not an uncommon finding in normal young children

Idiopathic Thrombocytopenic Purpura Idiopathic thrombocytopenic purpura may be suggested by the appearance of petechiae purpura and hemorrhagic manifestations However bone marrow aspiration reveals a conspicuous increase of megakaryocytes whereas they are suppressed in patients with leukemia In patients with thrombocytopenic purpura severe anemia is absent and the marrow reveals normal granulopoiesis and erythropoiesis in contrast to the anemia and infiltration by immature cells in patients with leukemia

Reticuloendotheliosis In reticuloendotheliosis in infants not associated with characteristic skin lesions sudden hematemesis and melena may occur with a diminution in the number of platelets Bizarre reticuloendothelial cells occasionally may be identified in the bone marrow and skin scrappings[69] as compared with the marked invasion of both the peripheral blood and marrow by abnormal cells in patients with leukemia

Aplastic Anemia The most frequent pancytopenic condition to be confused with leukemia is aplastic anemia In patients with aplastic anemia the bone marrow shows a progressive decrease in the myeloid elements nucleated red cells and megakaryocytes In leukemia except in the hypoplastic stage induced by chemotherapy or the infrequent spontaneous remission the bone marrow and peripheral blood are infiltrated by immature cells

Neuroblastoma The bone marrow in patients with neuroblastoma of the type referred to as sympathicoblastoma is sometimes sufficiently infiltrated by clusters of undifferentiated cells to suggest leukemia [0] The identification of pseudorosettes with cells arranged about a central mass of fibrils in patients with neuroblastoma separates the two conditions morphologically Also the peripheral blood of patients with neuroblastoma is free from abnormal cells Other clinical features such as the presence of an abnormal mass with displacement of the kidney and focal areas of calcification are indicative of neuroblastoma The roentgenogram cannot be employed for differentiation since leukemia of the skeleton closely resembles metastatic sympathicoblastoma [43]

Infectious Mononucleosis Infectious mononucleosis can be differentiated by the absence of significant anemia thrombocytopenia and close examination of the blood smear Lymphadenopathy and splenomegaly may be slight to moderate similar to early cases of acute leukemia Sore throat may be present in patients

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Exophthalmos due to retrobulbar hemorrhage or infiltration of the bones about the orbit is to be differentiated from chloroma which it closely resembles Retinal hemorrhages are common and occasionally extensive and may be associated with impaired vision or actual blindness Although these hemorrhages are ordinarily a valuable sign of intracranial bleeding they may be found in leukemic patients without other signs of nervous system involvement[98]

The cranial nerves especially the sixth and seventh may be infiltrated and involvement is diagnosed by specific neurologic signs and symptoms[9]

Increased intracranial pressure is a serious prognostic sign and is due to leukemic infiltration primarily of the meninges and to a lesser extent of the cerebral parenchyma It may be asymptomatic but is frequently accompanied by severe headache vomiting meningismus papilledema drowsiness and coma The cerebrospinal fluid is clear under increased pressure the protein and cell count are elevated and microscopic examination reveals blast forms and a low or normal concentration of sugar Roentgenograms of the skulls of the younger children show separation of the sutures It has been suggested[94] that there has been a greater incidence of this complication in patients treated with the hormones and chemotherapy than in previously untreated patients According to this premise the brain is attacked later than are the other organs so that prolonged survival permits the eventual involvement of the central nervous system There is the possibility also that such agents as 6 mercaptopurine[4] and amethopterin cross the blood brain barrier in greatly reduced amounts even though the disease is under control elsewhere The latter feature should be emphasized namely that intracranial complications frequently occur while the peripheral blood and bone marrow are in a complete hematologic remission

Leukemic meningitis occasionally simulates the bacterial form with cloudy fluid and decreased sugar content but examination of the cells reveals its leukemic etiology[3]

Differential Diagnosis Usually leukemia in childhood is diagnosed without difficulty after examination of the peripheral blood and bone marrow In equivo cal cases leukemia must be differentiated from disorders in which overlapping occurs in some aspect of the hematologic picture The most important feature of the diagnosis of childhood leukemia is the critical evaluation of the clinical picture so that leukemia is suspected and the peripheral blood and bone marrow are examined

count, and absence of splenomegaly in patients with the leukemoid reaction are contrasted with the severe anemia thrombocytopenia and enlarged spleen in those with leukemia In fulminating cases of congenital leukemia the differentiation is often made only at postmortem examination when tissue infiltration with white blood cell precursors reveals the leukemic nature of the disease In the patient with acute leukemia also there is a gap (hiatus leukemicus) between the myeloblast, lymphoblast or promyelocyte and the polymorphonuclear neutrophil which is not present in the patient with a leukemoid reaction

REFERENCES

1 Aisner M and Hoxie T B Bone and Joint Pain in Leukemia Simulating Acute Rheumatic Fever and Subacute Bacterial Endocarditis New England J Med. 238 733 1948

2 Abinklov J R and Hatoff A Acute Lymphatic Leukemia and Pregnancy Am J Dis Child 72 202 1946

3 Anderson R C Familial Leukemia A M A Am J Dis Child 81 313 1951

4 Anderson R C and Hermann, H W Leukemia in Twin Children J A M A 158 652 1955

5 Bassen F A and Kohn, J L Multiple Spontaneous Remissions in a Child With Acute Leukemia the Occurrence of Agranulocytosis and Aplastic Anemia in Acute Leukemia and Their Relationship to Remissions Blood 7 37 1952

6 Baty J M and Vogt E C Bone Changes of Leukemia in Children Am J Roentgenol 34 310 1935

7 Bedwell, G A and Dawson A M Chronic Myeloid Leukemia in a Child Presenting as Acute Polyarthritis Arch Dis Childhood 29 78 1954

8 Berlin N I Lawrence J H and Lee H C The Pathogenesis of the Anemia in Chronic Leukemia Measurement of the Life Span of the Red Blood Cell With Glycine-2C14 J Lab & Clin Med 44 860 1954

9 Bernhard W G Gore I and Kilby R A Congenital Leukemia Blood 6 990 1951

10 Bierman H R Aggeler P M Thelander H Kelly K H and Cordes F L Leukemia and Pregnancy Problem in Transmission in Man J.A M A 161 220 1956

11 Bierman H R Crile M Dod K S Kelly K H Petrakis N L White L P and Shimkin M B Remissions in Leukemia in Childhood Following Acute Infectious Disease Cancer 6 591 1953

12 Burge R F Jenks A L Jr and Davis S K Spontaneous Remission in Acute Leukemia Report of a Case Complicated by Eclampsia J A M.A 140 589 1949

13 Block M Jacobson L O and Bethard W F Preleukemic Acute Human Leukemia J A M A 152 245 1953

13a Brescia M A Santora E and Sarnatora V F Congenital Leukemia J Pediat 55 35 1959

14 Brown G M Elliott S M and Young W A Haemolytic Factor in Anaemias of Lymphatic Leukaemia J Clin Invest 30 130 1951

15 Brown, W M C and Abbatt, J D The Incidence of Leukaemia in Ankylosing Spondylitis Treated With X rays Lancet I 1283 1955

16 Burchenal J H Experimental Studies on the Relation of Pregnancy to Leukemia Am J Cancer 39 309 1940

17 Burchenal J H Murphy M L and Tan C T C Review Article Treatment of Acute Leukemia Pediatrics 18 643 1956

18 Carmel W J Minno A M and Cook W L Jr Eosinophilic Leukemia With Report of a Case A M A Arch Int Med 67 260 1951

19 Castle W B Meyer O and Clew W B Negative Results of Treatment of Chronic Myelogenous Leukemia as a Deficiency Disease Proc Soc Exper Biol & Med 32 660 1935

with both conditions but not to the extent found in those with the pharyngeal type of infectious mononucleosis

The serum of patients with infectious mononucleosis usually has a high titer of agglutinins for sheep cells The elevated heterophil antibody titer encountered occasionally in patients with leukemia and other neoplastic diseases can be shown to be normal Forssman antibodies by means of an absorption test (complete ab sorption by guinea pig kidney) [91]

The atypical mononuclear cell in patients with infectious mononucleosis pre sents morphologic features distinct from those of the leukemic cell Although the occasional Downey type III cells may resemble the lymphoblast the appearance of significant numbers of characteristic atypical cells points to a diagnosis of infectious mononucleosis In doubtful cases bone marrow aspiration will exclude leukemia but this is rarely necessary

Acute Infectious Lymphocytosis Acute infectious lymphocytosis occurs spo radically as well as in epidemic form and is characterized by a hyperleukocytosis due to an increase in normal small lymphocytes The mildness of the clinical course the absence of anemia thrombocytopenia and the lack of enlargement of spleen and lymph nodes serve to distinguish this condition from acute leukemia Bone marrow examination fails to reveal the characteristic cell pattern found in patients with leukemia The blood picture of patients with acute infectious lym phocytosis however may closely resemble that of those with chronic lymphocytic leukemia by the extreme elevation of white cells and the preponderance of small mature lymphocytes The age incidence of the two diseases is very different since chronic lymphocytic leukemia is a disease of older persons rarely seen in those under 40 years of age

Rheumatic Fever and Rheumatoid Arthritis Leukemic patients with low grade fever slight anemia leukopenia cardiac murmur and migratory bone and joint pain simulate the clinical and hematologic pictures in patients with rheumatic fever and rheumatoid arthritis [1 89] The diagnosis of leukemia is further obscured for a time at least by the absence of enlarged lymph nodes and spleen and of a clear cut blood picture The presence of polyarthritis with the associated signs of local inflammation provides additional evidence of the possibility of rheumatic disease [58] Suspicion as to the true nature of the disease should be aroused by the failure of the patient to respond to salicylates and by a favorable response to transfusion Destructive changes of the skeleton observed by roentgenography exclude rheumatic fever and direct attention to leukemia as the underlying dis ease Close examination of the blood smear despite the paucity of white cells usually reveals an occasional cell which can be identified as being leukemic in origin Bone marrow aspiration subsequently confirms the diagnosis of acute leukemia

Leukemoid Reactions The differentiation between leukemia and a leukemoid reaction may be difficult with respect to congenital leukemia and sepsis in the newborn infant and cases of chronic myelogenous leukemia in later childhood Confusion exists particularly when the blood and bone marrow show increased numbers of myelocytes and transitional forms including metamyelocytes and seg mented and nonsegmented polynuclear cells The milder anemia normal platelet

47 Biological Effects of Atomic Radiation Summary Reports From Study by National Academy of Sciences National Research Council, 1956
48 Medical Research Council Hazards to Man of Nuclear and Allied Radiations London, 1956 Her Majesty s Stationery Office
49 Kalayjian B S Herbut, P A and Erf L A The Bone Changes of Leukemia in Children *Radiology* 47 223 1946
50 Kaplan, H S On the Etiology and Pathogenesis of the Leukemias a Review Cancer Res 14 535 1954
51 Keith H M Chronic Myelogenous Leukemia in Infancy Report of a Case Am J Dis Child 69 366 1945
52 Kelsey W M, and Anderson, D H Congenital Leukemia, Am J Dis Child 58 1268 1939
53 Kirschbaum, A Recent Studies in Experimental Mammalian Leukemia, Yale J Biol & Med 17 163 1944 1945
54 *Koler R D, Seaman A J Osgood E E and Vanbellinghen, P Myeloproliferative Diseases Diagnostic Value of the Leukocyte Alkaline Phosphatase Test, Am J Clin Path.* 30 295 1958
55 Krivit W and Good, R A The Simultaneous Occurrence of Leukemia and Mongolism Report of 4 Cases A.M.A J Dis Child 91 218 1956
56 Krivit, W and Good, R A Report of a Nationwide Survey A.M.A *J Dis* Child 94 289 1957
57 Leonard B J and Wilkinson J F In Wilkinson John F (*editor*) The Acute Leukemias in Modern Trends in Blood Diseases New York, 1955 Paul B Hoeber Inc
58 Lewis E B Leukemia and Ionizing Radiation, Science 125 963 1957
59 MacMahon, B and Clark D Incidence of Common Forms of Human Leukemia, Blood 11 871 1956
60 Mallory T B Gall E O and Brickley W J Chronic Exposure to Benzene (Benzol) Pathologic Results Indust Hyg & Toxicol 21 355 1939
61 March H C Leukemia in Radiologists in 20-Year Period, Am J M Sc 220 282, 1950
62 Meacham G C and Weisberger A S Early Atypical Manifestations of Leukemia, A M A Arch Int Med 41 780 1954
63 Merrit D H and Harris J S Mongolism and Acute Leukemia, Arch Dis Childhood 92 41 19 6
64 Miller F R and Turner D L The Action of Specific Stimulators on the Hematopoietic System *Am J M Sc* 205 148 1943
65 Mills L C Boshton, B F Greene S A and Moyer J H. Septic Arthritis as a Complication of *Orally Given Steroid Therapy J A M.A.* 164 1310 1957
66 Moloney W C Leukemia in Survivors of Atomic Bombing New England J Med. 253 88 1955
67 Moloney W C and Kastenbaum M A Leukemogenic Effects of Ionizing Radiation on Atomic Bomb Survivors in Hiroshima City Science 121 308 1955
68 Moloney W C and Lange R D Leukemia in Atomic Bomb Survivors Observations on Early Phase of Leukemia *Blood* 9 663 1954
68a Moore E W Thomas L B Shaw R K and Freireich E J The Central Nervous System in Acute Leukemia A M A Arch Int Med 105 451 1960
69 *Moore T D A Simple Technique for the Diagnosis of Nonlipid Histiocytosis* Pediatrics 19 438 1957
69a *Nathan D J and Sanders M Manifestations of Acute Leukemia in the Parenchyma of* the Lung New England J Med 252 797 1955
70 *Ohme J* Jansen W and Hagitte C Leukamie im Kindesalter Beitrage zur Morphologie Klinik Pathophysiologie und Therapie Leipzig, 1958 Georg Thieme Verlag
71 Pearson, H A and *Diamond L K Chronic* Monocytic Leukemia in Childhood, J Pediat 53 259 1958
72 Perry S Coagulation Defects in Leukemia, J Lab & Clin Med 50 229 1957
73 Pierce M The Acute Leukemias of Childhood Pediat Clin North America 4 497 1957

20 Christenson W N Ultmann J E and Mohos S C Disseminated Neuroblastoma in an Adult Presenting the Picture of Thrombocytopenic Purpura Blood 11 273 1956
21 Cooke J V The Incidence of Acute Leukemia in Children J A M A 119 547 1942
22 Cooke J V Acute Leukemia in Twins J A M A 152 1028 1953
23 Cooke J V Chronic Myelogenous Leukemia in Children J Pediat 42 537 1953
24 Cooke J V The Occurrence of Leukemia Blood 9 340 1954
25 Cooperberg A A and Neiman G M A Fibrinogenopenia and Fibrinolysis in Acute Myelogenous Leukemia Ann Int Med 42 706 1955
25a Craddock C G The Physiology of Granulocytic Cells in Normal and Leukemic States Am J Med 28 711 1960
26 Cramblett H G Friedman J L and Najjar S Leukemia in an Infant Born of a Mother With Leukemia New England J Med 259 727 1958
26a Cronkite E P Moloney W and Bond V P Radiation Leukemogenesis Am J Med 28 673 1960
27 Cross F S Congenital Leukemia Report of Two Cases J Pediat 24 191 1944
28 Dale J H Jr Leukemia in Childhood a Clinical and Roentgenographic Study of Seventy Two Cases J Pediat 34 421 1949
29 Dameshek W and Gunz F W Diagnostic and Therapeutic X ray Exposure and Leukemia J A M A 163 838 1957
30 Diamond L K and Luhby L A The Pattern of Spontaneous Remissions in Leukemia in Childhood a Review of 26 Remissions in 300 Cases Am J Med 10 236 1951
30a Dameshek W and Schwartz R S Leukemia and Auto-immunization Some Possible Relationships Blood 14 1151 1959
31 Ellerman V and Bang O Experimentelle Leukamie bei Huhern Zentralbl Bakt 46 595 1908
32 Evans T S and Nesbit R R Eosinophilic Leukemia Report of Case With Autopsy Confirmation Review of Literature Blood 4 603 1949
32a Firkin B and Moore C V Clinical Manifestations of Leukemia Recent Contributions Am J Med 28 764 1960
33 Folley J H Borges W and Yamawaki T Incidence of Leukemia in Survivors of the Atomic Bomb in Hiroshima and Nagasaki Japan Am J Med 13 311 1952
34 Furth F Recent Studies on the Etiology and Nature of Leukemia Blood 6 964 1957
35 Gilbert E F and Rice E C Neurologic Manifestations of Leukemia Report of Three Cases Simulating Acute Bacterial Meningitis Pediatrics 19 801 1957
36 Gilbert E F Rice E C and Lechaux P A Renal Function in Children With Leukemia A M A J Dis Child 93 150 1957
37 Gilliam A G Age Sex and Race Selection at Death From Leukemia and Lymphomas *Blood 8 693 1953*
38 Gould W R Innes J and Robson H N Survey of 647 Cases of Leukemia Brit M J 1 585 1953
39 Gray J D and Shaw S Eosinophilic Leukaemia and Familial Eosinophilia Two Illustrative Cases Lancet 2 1131 1949
40 Gross L Is Leukemia Caused by a Transmissible Virus? A Working Hypothesis Blood 9 557 1954
41 Gunz F and Dameshek W Chronic Lymphocytic Leukemia in a Family Including Twin Brothers and a Son J A M A 164 1323 1957
42 Hamilton L and Elion G B The Fate of 6-Mercaptopurine in Man Ann New York Acad Sc 60 304 1954
43 Hansman C F and Girdany B R The Roentgenographic Findings Associated With Neuroblastoma J Pediat 51 621 1957
44 Hird A J Mikulicz s Syndrome Brit M J 2 416 1949
45 Hyman G A Studies on Anemia of Disseminated Malignant Neoplastic Disease The *Hemolytic Factor* Blood 9 911 1954
46 Ingalls T H Etiology of Mongolism Epidemiologic and Teratologic Mechanism Am J Dis Child 94 147 1947

99 Wetherley Mein G Epstein I S Foster W D and Grimes A J Mechanisms of Anaemia in Leukaemia Brit J Haemat 4 281 1958
100 Williams M J Myeloblastic Leukemia Preceded by Prolonged Hematologic Disorder Blood 10 502 1955
101 Wiltshaw E and Moloney W C Histochemical and Biochemical Studies on Leukocyte Alkaline Phosphatase Activity Blood 10 1120 1955
102 Wolfson I N Groce E J and Fite F K Acute Leukemia With Rupture of the Spleen as the Initial Symptom New England J Med 251 735 1954
103 Yaguda A and Rosenthal N The Relation of Trauma to Leukemia Am J Clin Path 9 311 1939

74 Pierce M I Leukemia in the Newborn Infant J Pediat 54 691 1959
75 Poncher H G Weir H F and Limarzi L R Chronic Myelogenous Leukemia in Early Infancy Case Report J Pediat 21 73 1942
76 Ross J F Crockett C L Jr and Emerson C P Mechanism of Anemia in Leukemia and Malignant Lymphoma J Clin Invest 30 668 1951
77 Sandberg A A Cartwright G E and Wintrobe M M Studies on Leukemia Uric Acid Excretion Blood 11 154 1956
78 Schunk G J and Lehman W L Mongolism and Congenital Leukemia J A M A 155 250 1954
79 Schwab R S and Weiss S The Neurologic Aspect of Leukemia Am J M Sc 189 766 1935
80 Schwartz E E and Lipton F E Factors Influencing the Incidence of Leukemia Special Consideration of the Role of Ionizing Radiation Blood 13 845 1958
81 Schwartz S O and Schoolman H M The Etiology of Leukemia the Status of the Virus as Causative Agent a Review Blood 14 279 1959
82 Shimkin M B Mortality From Leukemia and Lymphoma in the United States Sixth International Congress of the International Society of Hematology Boston 1956
83 Silver R T Beal G A Schneiderman M A and McCullough N B The Role of the Mature Neutrophil in Bacterial Infections in Acute Leukemia Blood 12 814 1957
84 Silver R T Utz J P Frei E and McCullough N B Fever Infection and Host Remission in Acute Leukemia Am J Med 24 25 1958
85 Silverman F N The Skeletal Lesions in Leukemia Am J Roentgenol 59 819 1948
86 Simpson C L and Hempelmann L H Association of Tumors and Roentgen Ray Treatment of Thorax in Infancy Cancer 10 42 1957
87 Simpson C L Hempelmann L H and Fuller L M Neoplasia in Children Treated With X rays in Infancy for Thymic Enlargement Radiology 64 840 1954
88 Smith C H Leukopenic Myeloid Leukemia Associated With Arthritis Am J Dis Child 45 123 1933
89 Smith C H Leucemia in Childhood With Onset Simulating Rheumatic Disease J Pediat 7 390 1935
90 Southam C M Craver L F Dargeon H W and Burchenal J H Study of Natural History of Acute Leukemia With Special Reference to Duration of Disease and Occurrence of Remissions Cancer 9 39 1951
91 Southam C M Goldsmith Y and Burchenal J H Heterophile Antibodies and Antigens in Neoplastic Diseases Cancer 4 1036 1951
92 Stewart A Webb J Giles D and Hewitt D Malignant Disease in Childhood and Diagnostic Irradiation in Utero Lancet 2 447 1956
93 Sturgis C C Some Aspects of the Leukemia Problem J A M A 150 1551 1952
94 Sullivan M P Intracranial Complication of Leukemia in Children Pediatrics 20 757 1957
94a Valentine W N The Metabolism of the Leukemic Leukocyte Am J Med 28 699 1960
94b Syverton J T and Ross J D The Virus Theory of Leukemia Am J Med 28 683 1960
94c Troup S B Swisher S N and Young L E The Anemia of Leukemia Am J Med 28 751 1960
95 Valentine W N and Beck W S Biochemical Studies on Leucocytes Phosphatase Activity in Health Leucocytosis and Myelocytic Leucemia J Lab & Clin Med 38 39 1951
96 Videbaek A Heredity in Human Leukemia and Its Relation to Cancer London 1947 H K Lewis & Co Ltd
97 Virchow R Weisses Blut und Milztumoren Medicinische Zeitung Berlin 15 157 163 167 1846 (erste) 16 9 15 1847 (zweiter)
98 Wells C E and Silver R T The Neurologic Manifestations of the Acute Leukemias a Clinical Study Ann Int Med 46 439 1957

in the form of transfusions of blood derivatives and antibiotics are directed respectively toward correction of the anemia and bleeding tendencies and treatment of infection

Chemotherapeutic Agents—Antimetabolites [4] The antimetabolites include the folic acid antagonists Aminopterin and amethopterin and the purine antagonist 6 mercaptopurine

Principle of the antagonist (analogues antimetabolites) It has been well established that structurally related compounds may be biologically antagonistic Antimetabolites in use in the treatment of leukemia are compounds whose chemical structure differs only slightly from a vitamin an amino acid or a precursor of nucleic acid These substances are so closely related chemically that they replace the metabolite in its particular enzyme system Thus folic acid analogues combine with folic acid systems because of their similar chemical structure In view of the structure not being the same as that of the metabolite the reaction ceases and the enzyme system and in this case the utilization of folic acid is blocked [36 37] For example synthetic analogues of many vitamins which inhibit the growth of organisms have been prepared Woods[3] originally observed that the bacteriostatic action of sulfanilamide was reversed by the chemically related para aminobenzoic acid These observations led also to the concept that the analogues exhibit their effects by producing signs of deficiency of the metabolite which they resemble structurally

The advent of potent antimetabolites which affect the synthesis of nucleic acid has focused considerable interest on its structure The nucleic acids are composed of relatively simple primary units phosphate the sugars ribose or deoxyribose the purines and the pyrimidines The building materials involved in purine synthesis are glycine carbon dioxide ammonia and certain one carbon compounds such as formate Folic acid is quite generally involved in one carbon unit metabolism and it appears to be specifically concerned with the synthesis of the purines It is assumed that by virtue of this fact the antifolic acid compounds exert their growth inhibitory action Thus nucleic acid synthesis might be interfered with by the use of folic acid antagonists (to block one carbon fragment incorporation) or by the use of purine or pyrimidine analogues (to block interconversion reactions and incorporation)

Folic acid antagonists Folic acid (PGA) a vitamin which occurs in nature and which is also synthetized consists of pteridine para aminobenzoic acid and glutamic acid Since it is essential for the growth of erythroid and myeloid cells of the bone marrow megaloblastosis results when it is deficient In addition to its specific action upon megaloblastic marrow folic acid exerts many other nutritional effects and participates in specific metabolic functions frequently in conjunction with vitamin B_1 Of basic importance is the fact that folic acid is essential for the growth of certain types of leukemic cells and with its derivatives plays an essential role in the biosynthesis of nucleic acid Citrovorum factor (folinic acid Leucovorin) is a derivative of and probably the active form of folic acid Conversion of folic acid to the citrovorum factor is enhanced in vivo and in vitro by ascorbic acid

Certain compounds biologically antagonistic to folic acid have the property

CHAPTER 23

Leukemia—Treatment

The aim of therapy in patients with leukemia is the eradication of the leukemic process in the blood and tissues and the restoration of hematopoiesis to normal No curative agent or method has as yet been discovered which permanently controls the disease Although it is invariably fatal substantial gains have been made in achieving remission and in prolonging life especially in patients with acute leukemia of childhood

Treatment The principal therapeutic agents in current use in childhood leukemia are as follows

1 Chemotherapeutic agents
   - A Antimetabolites—inhibitors of cellular metabolism (probably at nucleic acid level)
     - (1) Folic acid antagonists
       - (a) Aminopterin
       - (b) Amethopterin (Methotrexate)
     - (2) Purine antagonist
       - (a) 6 Mercaptopurine (Purinethol)
2 Cytotoxic agents
   - A Radiation therapy
   - B Busulfan (Myleran)
3 Hormones
   - A ACTH
   - B Cortisone hydrocortisone prednisone prednisolone
   - C Triamcinolone methylprednisolone dexamethasone
4 Supportive treatment
   - A Transfusions
   - B Antibiotics

Skillful management depends upon the judicial selection of these agents for administration alone or in combination Antileukemic therapy may be classified as specific and nonspecific In the specific group reliance is placed mainly upon ACTH and the adrenocortical steroids the folic acid antagonist amethopterin (Methotrexate) and the purine antagonist 6 mercaptopurine and to a lesser extent urethan Myleran and x ray therapy * Supportive or nonspecific therapy

* Still under trial and of a lesser value than those mentioned are analogues of 6 mercaptopurine such as 6 thioguanine and 6 chloropurine azaserine an analogue of serine which is used either alone or in combination with 6 mercaptopurine and demecolcin a colchicine derivative

of relapse which may precede clinical and peripheral blood changes Unless the number of leukemic cells in the bone marrow is very low relapses can occur quite rapidly Occasionally enlargement of the spleen heralds a relapse Repeated remissions may be obtained with amethopterin but each successive one is usually of shorter duration and more difficult to produce It is of interest that children can be maintained on amethopterin for prolonged periods in relatively good health with the bone marrow and blood showing partial relapse

TOXIC EFFECTS The initial symptoms of toxicity are loss of appetite and abdominal pains *The areas involved are the buccal mucous membrane gastro* intestinal tract and bone marrow Small shallow ulcers which are painful and which interfere with feedings may appear on the buccal mucosa of the lips and the cheeks and on the tongue At this point the dosage should be reduced or the drug discontinued otherwise serious ulceration of the gastrointestinal canal with massive hemorrhages develops Erythematous and hemorrhagic rashes of the scalp face neck and trunk nausea anorexia diarrhea, epistaxis and alopecia are other signs of toxicity These manifestations including bone marrow hypoplasia or aplasia usually ascribed to toxic effects of the drug may represent part of the leukemic process itself

A megaloblastic transformation of the bone marrow and peripheral macrocytosis represent true folic acid deficiency The bone marrow may show varying degrees of injury with respect to the red and white blood cell elements and the megakaryocytes Ultimately an aplastic bone marrow results with pancytopenia and bleeding unless the drug is withdrawn With prolonged administration reversibility is more difficult to obtain The simultaneous administration of ACTH *or the steroid hormones with reduced dosages of amethopterin may be effective in* controlling bleeding and in stimulating the aplastic bone marrow after the amethopterin has destroyed the blast cells Although folinic acid prevents the toxic effects of the folic acid antagonists it also interferes with their antileukemic action [3] Nevertheless with the development of severe toxicity the drug should be withdrawn and Leucovorin may be administered intramuscularly (3 mg dosage) The value of Leucovorin is open to question once flagrant toxic effects have appeared.

Purine antagonist—6 mercaptopurine (Purinethol) This analogue of the nucleic acid constituent adenine and the physiologic purine base hypoxanthine 6 mercaptopurine [18] has been found particularly effective in the treatment of acute leukemia *in children Its activity* in leukemias which are resistant to the antifols is indicative of an antipurine rather than an antifolic acid mode of action The drug interferes with the incorporation of purine into nucleic acid whereas the folic acid antagonists interfere with the synthesis of purines and pyrimidines involved in nucleic acid formation

DOSAGE The usual initial dose of 6-mercaptopurine is 2 5 mg per kilogram of body weight to the nearest 25 mg given once daily The approximate dose is 50 mg in an average 5-year old child and in children of all ages the range is from 25 to 150 mg daily The drug has a specific leukopenic effect which is manifested in seven to ten days after administration although maximal benefits require three to eight weeks of treatment As with amethopterin 6-mercaptopurine may be

of inhibiting cell growth The folic acid antagonists that have been most widely used are Aminopterin and amethopterin (Methotrexate) Both involve a change in the folic acid molecule by the addition of an amino group in the 4 position of of the pteridine ring The administration of either of these antagonists in controlled doses prevents the utilization of folic acid Normal as well as leukemic tissues are affected by these agents but the development of rapidly growing immature cells is impaired more than that of the mature slowly growing cells The body therefore is placed in a state of folic acid deficiency insufficient to injure normal cells [3]

The analogues Aminopterin (4 aminopteroylglutamic acid) and amethopterin (4 amino N^{10} methylpteroylglutamic acid) appear to exert their antimetabolic effect by blocking the conversion of folic acid to citrovorum factor itself These folic acid derivatives enter into the synthesis of purines and pyrimidines and in blocking the activity of purines and pyrimidines nucleic acid formation is reduced and cell growth is retarded

In many instances it seems likely that the leukemic cells rapidly acquire resistance to the folic acid and other antagonists Thus experience has shown that eventually all patients ultimately become resistant to these compounds but the mechanism by which this is accomplished is not definitely established [10]

AMINOPTERIN The chemotherapeutic agent Aminopterin was among the first of the analogues of folic acid to be employed successfully in the treatment of patients with acute leukemia [8, 7] It is given in amounts of 0 5 to 1 mg daily orally or intramuscularly (at all pediatric age periods) Although substantial improvement and complete remissions have been obtained with this drug clinical experiences have demonstrated the superiority of amethopterin The latter possesses a much wider margin of safety and therefore is less prone to produce toxicity from the dosages used than is Aminopterin

AMETHOPTERIN (METHOTREXATE) The dosage of amethopterin ranges from 1 25 to 5 mg given orally once daily The usual dose is 1 25 mg for very small children and 2 5 mg daily for children over 2 years of age At this dosage treatment can be carried out for prolonged periods without toxic effects In some patients 5 mg is required to obtain a remission but at this level evidences of toxicity frequently appear The drug is rapidly absorbed and constant supervision is necessary to avoid serious complications Intermittent therapy is sometimes useful in children particularly those susceptible to toxic reactions The regimen of ten days with and four days without the drug has proved of value in our hands

A remission following the administration of folic acid antagonists generally takes three to eight weeks although satisfactory responses have been obtained within ten days to two weeks Remissions with this drug can be maintained for periods of several months to a year and longer by skillful management by either continuous or intermittent therapy Good clinical and hematologic remissions may be expected in 30 to 50 per cent of patients [3] Following a remission therapy is usually continued on a dosage adjusted to maintain a normal blood and bone marrow status without signs of toxicity During treatment the bone marrow should be examined at two to four week intervals to detect early signs

of relapse which may precede clinical and peripheral blood changes Unless the number of leukemic cells in the bone marrow is very low relapses can occur quite rapidly Occasionally enlargement of the spleen heralds a relapse Repeated remissions may be obtained with amethopterin but each successive one is usually of shorter duration and more difficult to produce It is of interest that children can be maintained on amethopterin for prolonged periods in relatively good health with the bone marrow and blood showing partial relapse

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DOSAGE The usual initial dose of 6 mercaptopurine is 2.5 mg per kilogram of body weight to the nearest 25 mg given once daily The approximate dose is 50 mg in an average 5-year-old child and in children of all ages the range is from 25 to 150 mg daily The drug has a specific leukopenic effect which is manifested in seven to ten days after administration although maximal benefits require three to eight weeks of treatment As with amethopterin 6 mercaptopurine may be

increased above the stated dosage when the response is inadequate especially in the later stages of the disease

The drug is discontinued temporarily at the first evidence of a precipitous drop in the white blood cell count especially in patients with marked leukocytosis since a continued drop often follows the cessation of therapy When the leukocyte count rises or becomes stabilized for several days with counts ranging from 1 000 to 1 500 white cells per cubic millimeter treatment is reinstituted The same program is followed with elevated but more moderate initial white blood cell counts Periodic bone marrow aspiration is useful in determining to what extent the peripheral leukopenia is associated with leukemic infiltration The proliferation of the megaloblastic series characteristically seen with folic acid antagonists does not occur with 6 mercaptopurine If the bone marrow is still heavily involved treatment can be resumed provided the blood count does *not fall below 1 000 white blood cells per cubic millimeter From this level the* white blood cell count usually rises again within a few days A rapid decrease in the white blood cells in patients with hyperleukocytosis occasionally results in hyperuricemia and impaired renal function

A suitable maintenance dose should be established depending upon the character of the response in the initial treatment As with amethopterin 6 mercaptopurine may be given continuously or intermittently following a remission Relapses usually occur within four to six weeks after treatment is stopped Good clinical and hematologic remissions were observed in 47 per cent of patients in one series of eighty seven children[3] The drug produces remissions in children with acute leukemia who have been previously untreated and in those in whom the disease has become resistant to amethopterin and the steroid hormones Clinical improvement with reduction in size of the liver spleen and lymph nodes accompanies the return to normal of the bone marrow and peripheral blood 6 Mercaptopurine is rapidly metabolized disappearing from the plasma almost completely in twenty four hours Only in a dosage higher than that usually prescribed does the drug pass the blood brain barrier in appreciable amounts[13]

TOXIC EFFECTS The chief toxic manifestations are hematologic with marked leukopenia and bone marrow aplasia The drug is less toxic to the mucosa of the gastrointestinal tract than are the folic acid antagonists Mouth lesions nausea vomiting anorexia and mild diarrhea occur exceptionally

6 Mercaptopurine combined with azaserine The combination of 6 mercaptopurine and azaserine has been subjected to clinical trial in the treatment of patients with acute leukemia following the demonstration of its synergistic therapeutic effect in experimental animals Azaserine an analogue of serine which also blocks nucleic acid synthesis is given orally in a dosage of approximately 2 5 mg per kilogram of body weight Ulceration of the tongue and buccal mucosa is an indication for temporary withdrawal of this drug alone It is later resumed at a lower dosage of 1 25 mg per kilogram of body weight together with the full dose of 6 mercaptopurine In one series complete remissions were obtained with an average duration of six months thus delaying the resistance to 6 mercaptopurine alone on the average of about two months In another and

more extensive study[104] the duration of the remissions obtained with the combined therapy was not significantly longer than when 6-mercaptopurine was used alone

Cytotoxic Agents The principle cytotoxic agents used in the treatment of childhood leukemia are radiation therapy and the drugs urethan and busulfan (Myleran)

Radiation therapy Roentgen ray irradiation finds a place in the treatment of *acute leukemia in childhood It is effective in relieving pressure symptoms and* pain due to enlargement of infiltrated organs and localized leukemic masses It is an excellent adjuvant in relieving severe headache in patients with acute leukemia and in intractable bone and joint pain and disability in those with any type of leukemia, especially in the infrequent chronic myelocytic form in children In patients with chronic myelocytic leukemia especially it reduces the leukocytic count and the number of immature forms The technique of treatment is highly specialized and requires a consideration of the advantages of local versus generalized irradiation As with the specific drugs treatment needs to be carefully controlled by regular hematologic examinations so as to avoid pancytopenia and aplastic bone marrow

Busulfan (Myleran)—use in patients with chronic myelocytic leukemia The search for a less toxic analogue of nitrogen mustard led to the discovery of a sulfonic acid ester busulfan This drug has been found to be invaluable in the treatment of chronic myelocytic leukemia [111-120] and of little or no value in other types of leukemia It has largely replaced irradiation by x ray and the mitotic inhibitors urethan (ethyl carbamate) and demecolcin *(a derivative of colchicine)* previously used in treatment of this type of leukemia

MODE OF ACTION Busulfan is myelotoxic and depresses normal and abnormal myeloid tissue It tends to act selectively on granulocytes but in large doses platelets red cells and lymphocytes are also depressed Reduction in the leukocyte count is the prominent feature and may be achieved in ten to fourteen days or delayed for three or four weeks The remission is accompanied by the correction of the anemia a reduction or even a disappearance of the immature myeloid cells from the blood, a decrease in the cellularity of the bone marrow a diminution in the size of the liver and spleen and pronounced subjective improvement Large daily doses may produce thrombocytopenia and serious bone marrow depression Toxic side effects are confined to the bone marrow and little if any other discomfort is encountered Prolonged remissions lasting six to twelve months have been reported in adults

DOSAGE Busulfan is available in 2 mg tablets The usual dosage is 4 mg daily until a maximum hematologic and clinical improvement is obtained Ad*ministration of the drug is continued until the white blood cell count drops to* approximately 10 000 per cubic millimeter Occasionally 6 mg per day is required to attain a remission However the amount given must be individualized especially in children for a precipitous decrease in the leukocyte count may develop necessitating withdrawal of the drug until the white blood cell count is stabilized The need for maintenance therapy is still debatable A small dose adjusted to the particular patient may be given to prevent elevation or

further reduction in the white blood cell count. Some resume therapy when there is a substantial rise in the leukocyte count.[34]

Radioactive phosphorus (P^{32}) and nitrogen mustard and its derivatives such as triethylene melamine (TEM), although effective in patients with chronic leukemias, have not been found to be particularly valuable in those with acute leukemias.

Hormones. The hormones used in the treatment of leukemia in childhood include ACTH, cortisone, hydrocortisone, prednisone, prednisolone, triamcinolone, methylprednisolone and dexamethasone.

Hematologic response in normal subjects. The blood response to the administration of ACTH in human subjects with unimpaired adrenal function is well known—within four hours of the intramuscular injection of 25 mg. of ACTH, there is an increase in circulating neutrophils and a decrease in lymphocytes and eosinophils.[17] The involution of lymphoid tissue and dissolution of lymphocytes previously reported with the adrenocortical hormones provided a basis for clinical trial of these hormones in human beings with leukemia. The mechanisms by which neutrophilia, eosinopenia and lymphocytopenia are produced are not entirely understood, nor has the mode of action in leukemia found a satisfactory explanation. The inhibition of incorporation of a structural analogue of thymidine (I^{131} labeled iododeoxyuridine) into DNA by cortisone may implicate the latter directly or indirectly in nucleotide metabolism.[11a]

Role in treatment of leukemia. Corticotrophin (ACTH) and the adrenocortical steroids (cortisone, hydrocortisone, prednisone and prednisolone) have proved particularly effective in the treatment of acute leukemia in childhood. In contrast to the folic acid antagonists and 6-mercaptopurine, which require three to eight weeks to produce a satisfactory response, these hormones act rapidly. When serious bleeding occurs and the patient's condition is otherwise precarious, the hormones are the treatment of choice.

Rapid and dramatic improvement has been brought about by these hormones in critically ill patients within several days of treatment, and complete remission has been noted in three to four weeks. Satisfactory clinical and hematologic remissions occur with the initial use of ACTH and the steroid hormones in 50 to 75 per cent of children with acute leukemia.[1, 3, 34] The response in patients with acute myeloblastic and monoblastic leukemia is much less favorable.

In the first weeks of successful hormone therapy there is a disappearance of fever, a return of appetite, greater activity, a subsidence of bone and joint pain, a lessening of hemorrhagic manifestations and a regression of enlarged lymph nodes and spleen. Within one week the white blood cell count decreases, the blast cells decrease in the blood and bone marrow, and the reticulocyte count rises. Platelets increase in number after the first week, and hemoglobin levels rise. Bone marrow hypoplasia is not infrequently observed during the second week of treatment. Erythroblastic and granulocytic cells make their appearance, and in many patients a complete remission is achieved by the third to fourth week of treatment. Remission is relatively short and in most patients extends from three weeks to three months, but it occasionally extends from six to

nine months without further treatment Successive remissions are less frequent, incomplete and of shorter duration

Types and dosage No definite dosage can be stated for hormonal therapy since the speed of response and signs of hypercorticism which limit its use are subject to individual variation The following schedule serves as a guide to treatment and is based upon dosages in current use In order to provide sustained hormonal effects the total daily dosage must be divided

ACTH ACTH is administered in 25 mg doses by continuous intravenous drip in a glucose infusion over an eight to twelve hour period Rarely is more than one treatment given daily Intramuscular injection of an aqueous preparation or preferably the gel of 60 to 80 units daily is given in two divided doses to children under 4 years of age and 80 to 120 units is given to older children[23] ACTH is rapidly absorbed following intravenous or intramuscular injection

CORTISONE. Cortisone is administered orally in a total daily dosage of 100 to 200 mg It is given in divided doses at six hour intervals It has been largely replaced by hydrocortisone

HYDROCORTISONE. Hydrocortisone (compound F) can be given orally intravenously or intramuscularly depending upon the urgency of treatment. It produces maximal physiologic effects when given by continuous intravenous drip Because its effects are terminated rapidly when intravenous administration is stopped it is necessary to supplement it with the oral or intramuscular preparation The intravenous dosage is 100 mg daily Total oral dosage is 60 to 80 mg in younger children and 80 to 120 mg in older children Hydrocortisone is given at six hour intervals in four divided doses

PREDNISONE AND PREDNISOLONE. Prednisone and prednisolone serve as initial treatment or to maintain the improvement instituted by ACTH or to replace the antimetabolites in the event of relapse They possess the advantage of rapid onset of action and prompt therapeutic effects These hormones are available in 5 mg tablets and are given at six hour intervals In young children the tablets are crushed in stewed fruit or apple sauce

These compounds are produced by subtle modification in chemical structure of cortisone and hydrocortisone respectively Thus endowed with a potency three to five times greater than that of the parent compounds they possess relatively marked decreases in electrolyte side effects In moderate dosage (approximately 1.5 mg per kilogram) therapy is not complicated by sodium retention and excessive potassium excretion which occur with the older corticosteroid agents Although these precautions therefore do not apply to prednisone and prednisolone in average therapeutic doses hypopotassemia sodium retention and elevated blood pressure may occur when massive or excessive amounts are given over prolonged periods and appropriate measures must be taken to obviate these side effects

Prednisone and prednisolone are administered orally in doses of 15 to 20 mg daily in children under 2 years of age and in doses of 20 to 60 mg daily in older

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Hydrocortisone succinate (Solu-Cortef Upjohn) which contains 100 mg. of hydrocortisone in each vial is particularly suitable for intravenous use especially in emergency situations when high levels of hydrocortisone are needed immediately

children The average effective dose in children over 2 years old totals 40 to 60 mg daily in divided dosage with the higher level in the first two to three days

These steroids are gradually withdrawn when the bone marrow returns to normal Following cessation of therapy the bone marrow is re examined at two- to four week intervals In our experience maintenance therapy with small doses of prednisone given daily or intermittently has not prolonged the remission

Under present trial are new synthetic corticosteroids as substitutes for prednisone and prednisolone These are triamcinolone (Aristocort and Kenacort)* methylprednisolone (Medrol)† and dexamethasone (Deronil and Decadron)‡ It is claimed that the newer compounds require smaller dosage and produce a lesser incidence of side effects The 4 mg tablet of Aristocort Kenacort and Medrol corresponds to the 5 mg tablet of prednisone so that if trial is contemplated the dosage should be adjusted on the basis of four fifths of Meticorten One 0 75 mg tablet of Decadron or Deronil replaces 4 mg of methylprednisolone or triamcinolone or 5 mg of prednisone or prednisolone

When a complete clinical and hematologic remission has been achieved no further specific therapy is given until a relapse is in evidence Since subsequent remissions following the administration of the hormones are progressively shorter other chemotherapeutic agents are employed In some patients amethopterin has been instituted following the remission with hormones

Cross resistance between the hormones and folic acid antagonists or purine antagonists does not occur so that a patient who has become resistant to any of these agents may still be expected to respond to the others

Massive hormone therapy Remissions have been reported in adults and to a lesser extent, in children with acute leukemia of various types morphologically differentiated and undifferentiated following massive doses of prednisone and prednisolone Dosages of 1 gm daily (occasionally higher) have been recommended until satisfactory improvement is obtained with a variable toxicity[16, 4] Since the results with amethopterin and 6 mercaptopurine and lower doses of steroids have been satisfactory in children the use of massive therapy may find a place in the terminally ill patient when all other possibilities and combinations of therapy have been exhausted

Toxic and side effects Serious toxic and side effects indicative of hypercorticism develop with prolonged and excessive administration They consist of acneiform eruption hirsutism and increased deposition of fat in various parts of the body giving the "moon face" and the "buffalo hump" effect on the back of the neck Edema osteoporosis glycosuria and elevated blood sugar a tendency toward infections and clinical chemical and electrocardiographic evidences of hypokalemia represent other diverse effects Encephalopathy and cerebral hemorrhage in patients with severe thrombocytopenia also necessitate a reduction or withdrawal of hormone therapy Severe hypertension however is a rare complication in childhood

Adrenocortical steroids combined with amethopterin and 6 mercaptopurine Either amethopterin or 6 mercaptopurine may be combined with the steroids

* Aristocort—Lederle Kenacort—Squibb
† Medrol—Upjohn
‡ Deronil—Schering Decadron—Merck Sharp & Dohme

in the event of bleeding phenomena persistent hypoplastic marrow signs of nervous system involvement headache and anorexia Prednisone is given in a dosage of 20 to 40 mg depending upon the age of the patient

Adjuvant treatment with the adrenocortical steroids Patients treated with adrenocortical hormones are maintained on a low salt diet with additional potassium *in the form of potassium chloride 0 3 gm given orally several times* daily This caution applies particularly to patients being treated with ACTH cortisone and hydrocortisone With the latter also sodium and potassium determinations should be carried out initially and repeated only for clinical indications With this group of newer steroids (prednisone etc) sodium retention is not significant excessive potassium loss is easily controlled by having the patient take potassium chloride tablets or foods rich in potassium such as orange juice grapefruit or bananas

Remissions—Criteria Therapeutic responses are classified as either complete or partial depending upon the extent to which the bone marrow peripheral blood and organ involvement revert to normal In accordance with the recently established *standards of the Leukemia Chemotherapy Cooperative Study Group A*[1] a complete remission includes the following criteria In the bone marrow there are reduction in the number of blasts to less than 10 per cent with lymphocytes to less than 20 per cent and essentially normal appearing granulopoiesis erythropoiesis and thrombopoiesis In the peripheral blood there are return to and maintenance for more than one month of hemoglobin greater than 11 gm per 100 ml for children under 15 years of age or 10 gm for infants under 2 years of age granulocyte levels in excess of 1 500 per cubic millimeter platelet counts greater than 100 000 per cubic millimeter and an absence of leukemic cells in the blood smear Clinically signs ascribable to leukemia should be absent Criteria of a partial remission and a relapse involve increasing clinical and *hematologic evidences of leukemic infiltration*

With satisfactory hematologic and clinical improvement the patient should return to complete health and activity appetite should be normal and no *overt toxic effects of drug therapy should be manifested*

Detailed Program of Treatment With the diagnosis of leukemia the following factors require consideration choice of chemotherapeutic agent in relation to type of leukemia choice of a drug with which to initiate treatment the need for blood transfusions and other supportive therapy and the question of hospitalization and of psychological factors especially with relation to the parents

Choice of Chemotherapeutic Agent in Relation to Type of Leukemia Experience has demonstrated that significant differences in the therapeutic effects of various chemotherapeutic agents depend upon the type of leukemia diagnosed Following are the chemotherapeutic agents of choice in the treatment of various types of leukemia

Acute stem cell (lymphoblastic) leukemia

1 ACTH
2 Adrenocortical steroids
3 Amethopterin (Methotrexate)
4 6-Mercaptopurine (Purinethol)

Eosinophilic leukemia
1 Adrenocortical steroids
2 6 Mercaptopurine (Purinethol) (less effective)

Acute myeloblastic promyelocytic and myelocytic leukemia and monocytic leukemia
1 6 Mercaptopurine (Purinethol)
2 Amethopterin (Methotrexate)
3 Adrenocortical steroids

Chronic myelocytic leukemia
1 Busulfan (Myleran)

The acute stem cell (lymphoblastic) leukemia responds to ACTH and the adrenocortical steroids amethopterin and 6 mercaptopurine Eosinophilic leukemia responds principally to the adrenocortical steroids and less favorably to 6 mercaptopurine Acute myeloblastic promyelocytic and myelocytic and monocytic leukemias respond poorly to the adrenocortical steroids and better to 6 mercaptopurine Following relapse with 6 mercaptopurine amethopterin is used next and steroids are reserved when other agents fail Chronic myelocytic leukemia responds to busulfan Although these recommendations are more or less specific it is frequently necessary to deviate from them to introduce another drug when the response is greatly delayed The antimetabolites may have to be pushed however to the point of inducing severe leukopenia before deciding on their ineffectiveness To accomplish this the dosage may have to be increased with a close watch for toxicity At present busulfan appears to be the drug of choice in the treatment of chronic myelocytic leukemia

With the exception of ACTH and the adrenocortical steroids the antileukemic agents enumerated are capable of injuring the bone marrow The fact that myelotoxicity responsible for bone marrow aplasia is also destructive to leukemic cells suggests that for an agent to be of value therapeutically it must also have myelotoxic properties[4] This fact must be kept in mind in prolonged treatment with a chemotherapeutic agent and emphasizes the need for periodic bone marrow examinations

Occasionally early in treatment the antimetabolites produce signs of toxicity of sufficient severity to warrant withdrawal of the drug These signs consist of anorexia listlessness fever extreme leukopenia and increased bleeding from the gums and other areas In such an event the adrenocortical steroids and fresh whole blood are valuable in tiding the patient over the period until the specific therapy is reinstituted It may be necessary in the sensitive patient to continue adrenocortical steroids in small dosage combined with the appropriate amount of amethopterin or 6 mercaptopurine

From the response in normal subjects ACTH and the adrenocortical steroids appear to be contraindicated in patients with acute granulocytic leukemias Although they possess the potentiality of producing exacerbations in patients with this type of leukemia they may be used to advantage at least temporarily in controlling hemorrhage and in restoring the well being of the patient

Choice of Drug With Which to Initiate Treatment The principal chemotherapeutic agents in current use in acute leukemia in childhood are amethopterin 6 mercaptopurine and ACTH and the adrenocortical steroids The practice

is to prescribe each drug in turn as resistance develops Except for the adreno cortical steroids the same drug with which a remission is achieved is continued *in smaller dosage for maintenance therapy*

In treating with either amethopterin or 6 mercaptopurine the dosage is in creased before shifting to the other class of antimetabolites Combination ther apy with any two or three of the agents is eventually resorted to in the later stages of the disease when there is no response to each separately since there is no cross resistance between them But there is no evidence that the combination of these drugs proves any more effective at any stage of the disease than each *given separately in influencing the length of the remission*

Amethopterin and 6 mercaptopurine produce longer remissions than the adren ocortical steroids but generally require three to eight weeks for a maximal re *sponse With both drugs satisfactory responses occasionally have been observed* within a week or ten days

With ACTH and the adrenocortical steroids remissions occur within one to three weeks but are shorter than with the antimetabolites *Occasionally* remis sions of seven and eight months have been observed without therapy following the initial use of prednisone Although each remission with the hormones be comes progressively shorter nevertheless they may be repeated following their initial use as a relapse develops Following the administration of adrenocortical steroids the length of the remission in individual patients may be so prolonged that it is common practice not to institute another drug until evidences of a re lapse are noted An alternative plan infrequently adopted is to introduce ame thopterin in full dosage just as soon as the initial remission with the adrenocortical hormones occurs The latter program requires long term evaluation with respect *to comfort to the patient and length of survival The steroids are employed to* control bleeding manifestations at any time during the disease regardless of the drug in current use

In the gravely ill child with other than stem cell or undifferentiated leukemia usually the granulocytic and monocytic types ACTH and the hormones are nevertheless administered These agents are supplemented by supportive treat *ment until the immediate emergency is over when the specific drug may be given*

With these guiding principles as a basis current practices in the treatment of the acutely ill child who is febrile and in whom hemorrhage is manifest has been *to initiate therapy with ACTH and the adrenocortical steroids* The drug is also indicated in the occasional patient with acute stem cell or undifferentiated leukemia with a hyperleukocytosis who is not critically ill

Since the white blood cell count frequently undergoes a precipitate drop with steroid treatment an alternate method in the relatively well child with marked elevation of leukocytes is to prescribe 6 mercaptopurine so as to avoid *too rapid dissolution of white blood cells leading to hyperuricemia* and kidney impairment In our own experience such untoward events have not occurred although cases of hyperleukocytosis are carefully controlled by blood uric acid and urea *nitrogen determinations In the majority* of patients with leukemia the clinical condition is fairly good and the white blood cells range from a leuko penia to a moderate leukocytosis The best results however depend upon early

Eosinophilic leukemia

1 Adrenocortical steroids
2 6 Mercaptopurine (Purinethol) (less effective)

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once by platelet rich plasma also obtained by plastic bag technique and given continuously unless the hemoglobin is reintroduced By assiduous application of this program of intensive and continuous therapy bleeding is usually controlled

It should be remembered that patients receiving multiple transfusions of whole blood may occasionally develop thrombocytopenia due to the formation of antibodies against platelets

Concentrated platelets in patients with severe bleeding have proved effective in producing temporary hemostasis[19] but in an emergency they are usually difficult to prepare As has already been stated ACTH and cortisone have been shown to increase capillary resistance[6] so that these hormones as well as prednisone are useful adjuncts in the control of bleeding It has been shown however that ecchymoses may occur in patients undergoing prolonged ACTH and steroid therapy[6, 8] The administration of ascorbic acid (200 to 300 mg daily) has therefore been recommended as supplementary treatment[4]

Bleeding from accessible areas is occasionally controlled by the local application of thromboplastin Gelfoam or packs saturated with a mixture of thrombin and Adrenalin

Unless there is associated hemorrhage transfusions are not required until hemoglobin levels decrease to 7 or 8 gm per 100 ml The dosage of whole blood packed cells and plasma for the different age groups are listed in Chapter 7

Antibiotics Broad spectrum antibiotics are frequently given routinely whenever adrenocortical steroids are administered Prolonged administration of ACTH and of the steroids is known to inhibit the inflammatory process with the result that serious infection can develop without the usual clinical signs and symptoms Advocates of this policy also cite the fact that the leukemic patient is prone to develop infections as a result of the depletion of myeloid elements in the blood and bone marrow

The difficulties with routine use of the antibiotics with the steroids and the antimetabolites are the possibility of eventual resistance to specific pathogenic organisms and the development of widespread and fulminating monilial infection A more realistic approach is to administer antibiotics when infection is present This is often manifested in the initial stages of the disease and at varying periods subsequently

With severe and prolonged infection when sepsis is suspected the choice of an effective antibiotic depends upon bacteriologic methods It is not always possible however to isolate the causative organism in order to determine its susceptibility to antibiotics by in vitro tests The tetracyclines have been effective and are given orally in a dosage of 20 to 40 mg per kilogram of body weight When bleeding is minimal injections can be given intramuscularly Penicillin in combination with streptomycin is administered once or twice daily each in a total dosage of 300 000 to 600 000 units and 20 to 40 mg per kilogram of weight respectively Chloramphenicol given orally in a dosage of 40 mg per kilogram often has been found singularly effective in infections refractory to other antibiotics despite its known toxic effect on bone marrow It may be administered by drip intravenously in critically ill patients in a dosage of 100 mg per kilo

detection of the disease and control of its progress as vigorously as possible with antileukemic therapy

Since there is no urgency for a rapid control of the disease in the mild cases it is the custom to initiate therapy with the antimetabolites amethopterin or 6 mercaptopurine a low platelet count being no contraindication to their use Experience has shown that either antimetabolite may be given initially to the patient with the milder type of acute stem cell leukemia According to this plan ACTH and the adrenocortical steroids are kept in reserve for the periods when the antimetabolites can no longer control the disease

It is impossible to set forth absolute criteria for the administration of anti leukemic therapy except in the seriously ill child in whom ACTH and the adreno cortical steroid therapy is mandatory The individual response is so varied with each class of compounds that judgment must be exercised in the replacement of one drug by another and in relation to dosage Some clinicians prefer to postpone antileukemic therapy until the symptoms and clinical findings progress hoping thereby to conserve essential therapy and prolong the period of survival Most commonly therapy is initiated in all patients with leukemia as soon as the diagnosis is established In our clinic except in patients with the mildest form of the disease in whom antimetabolites are prescribed we have preferred to initiate treatment with prednisone Since a remission is predictable with this agent in at least 70 per cent of patients with acute stem cell leukemia within three to four weeks of treatment against a more prolonged period for the anti metabolites the parents are given an opportunity to orient themselves with regard to the seriousness of the disease and subsequent management within the initial period of hospitalization

Supportive Therapy Transfusions and antibiotics are the methods of suppor tive therapy chiefly relied upon in patients with leukemia

Transfusions Blood is administered to combat anemia and to control hemor rhage There is also evidence that the action of blood may not be confined solely to its capacity for raising hemoglobin but that it may also be responsible for an occasional remission This rare phenomenon noted after ordinary trans fusions has led to the hypothesis that normal blood contains an antileukemic substance More directly it has been shown that fresh blood rather than stored blood was effective in producing a decline in the total number of leukocytes without however modifying the differential count[3]

The hemoglobin value at which a transfusion is indicated varies with the associated signs and symptoms present in the patient Although the requirements for raising hemoglobin levels to correct anemia can be met by the administration of packed cells whole blood is often more desirable because thrombocytopenia is commonly present especially during chemotherapy Fresh whole blood ob tained in siliconized equipment or platelet rich plasma derived from such a sample has proved remarkably effective in the control of hemorrhage

For overwhelming and persistent hemorrhage from the nose mouth and gastrointestinal canal we have found that a routine of continuous treatment with whole blood and its products is beneficial Fresh blood collected in a plastic bag is given until the hemoglobin level is normal This is followed at

marrow material might conceivably generate normal blood elements The results thus far have been uniformly unsuccessful although questionable transient improvement has been cited [1] The collecting and storing of human marrow from living persons or cadavers for subsequent injection present formidable prob lems which are now in the process of solution The successful transplantation of human bone marrow would offer long sought for help in the treatment of patients with aplastic anemia leukemia and disseminated neoplastic disease

Laboratory Determinations In the patient who is hospitalized daily peri pheral blood counts are important to determine the effect on the leukocyte count and leukemic cells platelet and reticulocyte counts may be done periodically The bone marrow is examined at the outset and again at the end of the second and fourth weeks of initial treatment Subsequently peripheral blood counts may be carried out at weekly or biweekly intervals Bone marrow examinations are required if suspicious signs of relapse develop if marked leukopenia is noted in the peripheral blood or if the patient s condition deteriorates so that a change in therapy is contemplated

Blood urea nitrogen and uric acid estimations are necessary in the event of a precipitous fall in the white blood cell count with signs of obstructive uremia

Treatment of Nervous System Involvement Nervous system involvement has been noted with increasing frequency in children whose lives have been prolonged with chemotherapy [3] [1] Frequently neurologic symptoms occur while the peripheral blood and bone marrow are in remission Vomiting papilledema separation of the cranial sutures headache stiff neck and pleocytosis (lympho blasts) point to leukemic infiltration of meningeal and nerve tissues Irradiation to the brain or vertebral column to supplement chemotherapy has often been effective in relieving symptoms and in decreasing the cells in the spinal fluid ACTH or steroid hormones alone or in combination with amethopterin or 6 mercaptopurine in the usual or increased dosage have also been useful

Because of its poor penetration into the cerebrospinal fluid amethopterin should be given intrathecally [33] The dose is either 0 25 mg per kilogram of body weight every second to third day or 0 5 mg per kilogram every fourth to fifth day with an additional dose after the spinal cell count has returned to normal [1] The dose is given in 5 ml of spinal fluid or saline solution through a lumbar puncture at the level of the fourth and fifth lumbar vertebrae Usually three doses suffice but more may be required depending on the spinal fluid response During intrathecal therapy oral amethopterin is discontinued

Results of Treatment—Prognosis for Survival The beneficial effects of treat ment may be measured by a comparison of the duration of life in the prehormonal era and in the posthormonal and chemotherapeutic era In an analysis of cases[31] for the period in which no treatment was available 50 per cent of the children with acute leukemia given supportive therapy not including antibiotics expired within a period of approximately four months after the onset of the first definite symptoms The middle two thirds of patients survived from approximately two months to eight months and 10 per cent for as long as eleven months In a smaller series [3] 80 per cent treated with transfusions alone survived less than six months In another untreated group comprised of children and adults with

gram of body weight in conjunction with parenteral ACTH and hydrocortisone therapy

Hospitalization Except for the most seriously ill patients for whom hospitalization is urgent the average patient with leukemia can be managed on an ambulatory basis In patients with the milder disease for whom ACTH and the steroid hormones are chosen for initial treatment hospitalization is necessary because a precipitous drop in the white blood cell count frequently occurs soon after treatment is instituted necessitating adjustment of dosage

Whether the leukemic child with even the mildest form of the disease should be treated from the first on an ambulatory program is debatable In our experience an introductory period of hospitalization for about a week or ten days at the time of the diagnosis has proved extremely useful regardless of the chemotherapeutic agent that is chosen and the degree of severity It permits a more adequate compilation of data including roentgenographic studies on the basis of which a program of treatment is planned

When the antimetabolites are introduced initially severe myelotoxic effects may be manifested within the first two weeks of treatment in sensitive patients Hospitalization during this period offers an opportunity for institution of adjuvant treatment especially in patients with prolonged bleeding

More important is the opportunity which an initial hospital stay provides in helping parents adjust to the traumatic impact of a fatal childhood disease Frequent interviews with the attending physician help to acquaint parents with miscellaneous details concerning the nature of the disease which they are anxious to ascertain Following this hospitalization however ambulatory treatment is desirable unless serious clinical and hematologic relapses develop

Simplified Program of Treatment in Stem Cell (Lymphoblastic) Leukemia The plan most often followed in our clinic for patients with stem cell leukemia is to initiate treatment with prednisone until a clinical and hematologic remission is established During this period the bone marrow is examined weekly With a remission at approximately three to four weeks the child is discharged without further antileukemic therapy Strict attention is given subsequently to incipient signs of a relapse These include pallor fatigability loss of appetite bone pains chilly sensations easy bruising and beginning enlargement of the spleen At this time the bone marrow is re examined for confirmation of leukemic spread

With the relapse amethopterin or 6 mercaptopurine is administered With a subsequent relapse prednisone is reintroduced and within a few days either amethopterin or 6 mercaptopurine is substituted for the drug in previous use In the absence of signs of toxicity from the antimetabolite prednisone is gradually withdrawn

Bone Marrow Transplantation [21, 31] The demonstration that several species of animals given lethal doses of irradiation will recover after the infusion of normal bone marrow prompted trial of this procedure in human beings Leukemia particularly has been the object of energetic clinical experimentation

It is of interest that marrow injected intravenously seeks out the denuded marrow spaces and repopulates them Theoretically total body irradiation destroys the immunologic defenses so that heterotransplants of normal human bone

14 Haut A Altman S J Cartwright G F and Wintrobe M M The Influence of Chemotherapy on Survival in Acute Leukemia Blood 10 875 1955
15 Haut A Altman S J Wintobe M M and Cartwright G E The Influence of Chemotherapy on Survival in Acute Leukemia Comparison of Cases Treated During 1954 to 1957 With Those Treated During 1947 to 1954 Blood 14 828 1959
15a Heyn R M Brubaker C A Burchenal J H Cramblett H G and Wolff J A The Comparison of 6 Mercaptopurine With the Combination of 6 Mercaptopurine and Azaserine in the Treatment of Acute Leukemia in Children Results of a Cooperative Study Blood 15 350 1960
16 Hill J M Marshall G J and Falco D J Massive Prednisone and Prednisolone Therapy in Leukemia and Lymphomas in the Adult J Am Geriat Soc 4 627 1956
17 Hills A G Forsham P H and Finch C A Changes in the Circulatory Leukocytes Induced by Administration of Pituitary Adrenocorticotrophic Hormone (ACTH) in Man Blood 3:755 1948
18 Hitchings G H and Elion G B The Chemistry and Biochemistry of Purine Analogues Ann New York Acad Sc 60 195 1954
19 Klein E Toch R Farber S Freeman G and Fiorentino R Hemostasis in Thrombocytopenic Bleeding Following Infusion of Stored Frozen Platelets Blood 11 693 1956
20 Louis J Limarzi L R and Best W R Treatment of Chronic Granulocytic Leukemia With Myleran A M A Arch Int Med 97 299 1956
20a Mandel H G The Physiological Disposition of Some Anticancer Agents Pharmacol Rev 11 743 1959
21 Murphy M L Leukemia and Lymphoma in Children Pediat Clin North America 6 611 1959
22 Mercaptopurine and Nucleic Acid Synthesis Nutrition Reviews 13 247 1955
23 Pierce M I The Acute Leukemias of Childhood Pediat Clin North America p 497 May 1957
24 Ranney H M and Gellhorn A The Effect of Massive Prednisone and Prednisolone Therapy on Acute Leukemia and Malignant Lymphomas Am J Med 22 405 1957
25 Robson H N and Duthie J J R Capillary Resistance and Adrenocortical Activity Brit M J 2 971 1950
26 Robson H N and Duthie J J R Further Observations on Capillary Resistance and Adrenocortical Activity Brit M J 1 994 1952
27 Smith C H and Bell W R Aminopterin in Treatment of Leukemia in Children Serial Aspirations of Bone Marrow as a Guide to Management and Appraisal of Treatment Am J Dis Child 79 1031 1950
28 Stefanini M Ecchymoses Following Prednisone Therapy J A M A 164 1383 1957
29 Stefanini M and Martino N B Use of Prednisone in the Management of Some Hemorrhagic States New England J Med 254 313 1956
30 Thomas E D Lochte H L Jr and Ferrebee J W Irradiation of the Entire Body and Marrow Transplantation Some Observations and Comments Blood 14 1 1959
31 Tivey H Prognosis for Survival in Leukemias of Childhood Review of Literature and Proposal of Simple Method of Reporting Survival Data for These Diseases Pediatrics 10 48 1952
32 Wetherley Mein G and Cottom D G Fresh Blood Transfusion in Leukemia Brit J Haemat 2 25 1956
33 Whiteside J A Philips F S Dargeon H W and Burchenal J H Intrathecal Methotrexate Therapy in Children With Neurological Manifestations of Acute Leukemia A M A Arch Int Med 101 279 1958
34 Wintrobe M M Clinical Hematology ed 4 Philadelphia 1956 Lea & Febiger
35 Woods D D Relation of p Aminobenzoic Acid to Mechanism of Action of Sulphanilamide Brit J Exper Path 21 74 1940
36 Woolley D W A Study of Antimetabolites New York 1952 John Wiley & Sons Inc
37 Woolley D W Antimetabolites Science 129 615 1959

acute leukemia the figure of 50 per cent survival at four months is given[11]

In contrast to these groups is the effect on longevity of treatment with the antimetabolites ACTH and the adrenocortical hormones With this program of treatment 25 per cent still succumb within the first six months although a small number survive beyond two years [3] The increased survival in patients with the lymphoblastic type of leukemia which is prevalent in childhood has been emphasized especially in those patients with counts below 10 000 per cubic millimeter[1a] The results of treatment have been summarized as follows[1] for patients treated before folic acid antagonists were used the 50 per cent survival time was four to five months as compared with 9 2 months when folic acid antagonists and/or adrenocortical steroids were used and 12 5 months since 6 mercaptopurine has been added [1] Equally impressive is the striking clinical improvement in most patients which accompanies the hematologic remission induced by the newer therapy

REFERENCES

1 Atkinson J B Mahoney F J Schwartz I R and Hesch J A Therapy of Acute Leukemia by Whole Body Irradiation and Bone Marrow Transplantation From an Identical Normal Twin Blood 14 228 1959

2 Burchenal J H The Treatment of Leukemia Bull New York Acad Med 30 429 1954

3 Burchenal J H Murphy M L and Tan C T C Review Article the Treatment of Acute Leukemia Pediatrics 18 643 1956

3a Cramblett H G Recognition and Treatment of Intracranial Manifestations of Leukemia A M A J Dis Child 97 805 1959

4 Dameshek W The Outlook for the Eventual Control of Leukemia New England J Med 250 131 1954

5 Dameshek W Bone Marrow Transplantation a Present Day Challenge Blood 12 321 1957

6 Denko C W and Schroeder L R Ecchymotic Skin Lesions in Patients Receiving Prednisone J A M A 164 41 1957

7 Dougherty T F and White A Role of Pituitary Adrenotropic Hormone in Regulation of Lymphocytes and Other Cellular Elements of Blood Endocrinology 35 1 1944

8 Farber S Diamond L K Mercer R D Sylvester R F Jr and Wolff J A Temporary Remissions in Acute Leukemia in Children Produced by Folic Acid Antagonist 4 Aminopteroyl—Glutamic Acid (Aminopterin) New England J Med 238 787 1948

9 Ferrebee J W Atkins L Lochte H L Jr McFarland R B Jones A R Dammin G J and Thomas E D The Collection Storage and Preparation of Viable Cadaver Marrow for Intravenous Use Blood 14 140 1959

10 Fountain J R The Chemotherapy of Acute Leukemia a Review of its Present Status Edinburgh M J 61 69 1954

11 Galton D A G and Till M Myleran in Chronic Granulocytic Leukemia Lancet 1 425 1955

11a Gitlin D Commerford L and Hughes W L Inhibition of the Incorporation of Iododeoxyuridine Into DNA by Cortisone Society for Pediatric Research Paper No 10 30th Annual Meeting May 3 and 4 1960 Swampscott Mass

12 Haddow A and Timmis G M Myleran in Chronic Myeloid Leukemia Lancet 1 207 1953

13 Hamilton L and Elion G B The Fate of 6 Mercaptopurine in Man Ann New York Acad Sc 60 304 1954

13a Haurani F I Repplinger E and Tocantins L M Attempts at Transplantation of Human Bone Marrow in Patients With Acute Leukemia and Other Marrow Depletion Disorders Am J Med 28 794 1960

liver lymph nodes and bone marrow rather than to have aggregated as nodular masses as they do in multiple myeloma [4, 46] Hyperglobulinemia and occasionally Bence Jones proteinuria can sometimes be present [46] These cases are often difficult to differentiate from multiple myeloma which may also be accompanied by substantial numbers of plasma cells in the peripheral blood [5]

Bone Marrow Replacement and Leukoerythroblastosis Space occupying lesions of the bone marrow resulting from infiltration with foreign cells or an overgrowth of fibrous or bony tissue are associated with a heterogeneous group of diseases This process results in a chronic progressive anemia a low or occasionally greatly elevated leukocyte count and the presence of varying numbers of immature myeloid and erythroid cells in the peripheral blood [47] These conditions are accompanied by marked enlargement of the spleen and liver in which blood cells are produced which normally originate in the bone marrow The resulting blood disorders have been described under the title of myelophthisic or leukoerythroblastic anemia and since anemia need not necessarily be present the blood response may be termed leukoerythroblastosis

Leukoerythroblastosis is characterized by the presence of immature leukocytes of the myeloid series in the peripheral blood and nucleated red cells out of proportion to the degree of anemia It may be provoked by invasion of the *bone marrow by malignant cells of organs likely to metastasize to the bones* usually carcinomas Children with lymphosarcoma and Hodgkin s disease may develop this type of blood response The abnormal blood picture results from irritation or stimulation of normal adjacent marrow into excessive myeloid and erythropoietic activity

Myelofibrosis The syndrome of myelofibrosis is a myeloproliferative process involving the bone marrow accompanied by extramedullary hemopoiesis and splenic enlargement It occurs less frequently in children than in adults The process may be either focal or generalized or primary or secondary and is of varying grades of severity Myelosclerosis is characterized by increased production of fibrous (myelofibrosis) and bony tissue (osteosclerosis) Fibrosis and osteosclerosis of the marrow occur as a primary and idiopathic disease and in connection with such conditions as polycythemia in the course of the disease and in the "spent" phase leukemia Hodgkin s disease Gaucher s disease xanthomatosis tuberculosis, infections caused by chemical or physical agents and renal rickets [3] A diverse terminology has been applied to the syndrome such as myeloid metaplasia of the spleen leukoerythroblastic anemia osteosclerosis agnogenic myeloid metaplasia of the spleen and erythroleukemia (*Di Guglielmo s syndrome*)

Myelofibrosis is associated with slight to marked leukocytosis a leukemoid reaction with early granulocytes myelocytes and occasionally myeloblasts variation in red cell morphology with normoblasts and teardrop shaped erythrocytes [6, 34] anisocytosis poikilocytosis variable platelet levels circulating megakaryocytic fragments and tissue megakaryocytosis The hemoglobin level is usually low but may be normal Aspiration of a hypocellular bone marrow and difficulty in obtaining marrow at aspiration because of hardness of the bone indicate the presence of fibrous or other pathologic tissue Dry taps are fre

CHAPTER 24

Leukemia—Allied Disorders

There are a number of conditions of a leukemic nature that occur infrequently which resemble the more common forms clinically and are characterized by an abnormal cell of an unusual type Also allied to this unusual group of leukemias are the solid leukocytic neoplasms such as chloroma and the lymphomas in which the process is aleukemic in a few sites at the onset but which as it becomes generalized may simulate the typical blood and bone marrow picture of leukemia

Infrequent Types of Leukemia Among the unusual types of leukemia and extremely uncommon in children are the mast cell basophilic and plasma cell leukemias

Mast Cell Leukemia Mast cells which have been shown to produce the characteristic skin lesions in urticaria pigmentosa can occasionally infiltrate many viscera and supporting tissues Ellis [1] described the involvement of the skin bone marrow mesenteric lymph nodes spleen and liver in a 1 year old infant Bone marrow involvement is reflected in the roentgenogram by marked thickening and increased density especially of the frontal bone of the skull and coarsening of the trabeculations and thinning of the cortices in the small and large tubular bones [48] Conditions in which there is splenic involvement with a generalized visceral distribution of cells hepatosplenomegaly the appearance of mast cells in the peripheral blood anemia and at times thrombocytopenia have been classified as mast cell leukemia [0, 6, 60] The difficulty of separating the mast cell from the blood basophil is reflected in the patient in whom tissue and marrow mastocytosis is associated with blood basophilia The differentiation between the tissue mast cell and basophilic leukocyte is described elsewhere (Chapter 18)

Basophilic Leukemia Increased numbers of basophilic cells have been described in the course of chronic myelocytic leukemia In one instance of acute leukemia in a 5 month old infant mature basophilic leukocytes and myelocytes predominated [18] In another case in a young adult mature basophilic cells characterized the blood smear but were not associated with immature forms [8]

Plasma Cell Leukemia Cases of leukemia have been described in which plasma cells appeared in large numbers in the peripheral blood accompanied by anemia and leukocytosis These cells were found to have diffusely infiltrated the spleen

megakaryocytosis in the hematopoietic tissues or with myelosclerosis.[4] Extramedullary megakaryocytosis is less efficient in the formation of platelets than is the bone marrow and accounts for the discrepancy between the platelet count and the number of megakaryocytes in the metaplastic organs.[4] Whether the condition can be categorized as megakaryocytic leukemia in the absence of invasion of these cells into the blood stream is still controversial.[39]

Thrombocythemia Megakaryocytic hyperplasia of the bone marrow with markedly increased platelet counts (thrombocythemia) is regarded as a proliferative disorder of the bone marrow. Primary[46] and secondary types have been recognized. Secondary thrombocythemia occurs in association with an underlying condition such as carcinoma, myelogenous leukemia, polycythemia and Hodgkin's disease and following splenectomy. It has also been described as an idiopathic disease with prolonged bleeding time, venous thromboses, splenomegaly, spontaneous hemorrhage and a normal coagulation time. The cause of the hemorrhage and prolonged bleeding time is not known.[4]

Erythremic Myelosis (Di Guglielmo's Disease) Erythremic myelosis is a rare disease described by Di Guglielmo[16] which is characterized by erythropoietic hyperplasia within the bone marrow and extramedullary sites. Irregular remittent fever, splenomegaly proportionally greater than hepatomegaly, thrombocytopenia, granulopenia and anemia are associated with enormous numbers of erythroblasts in the peripheral blood which are basophilic, multinucleated or otherwise atypical.[36] Both the bone marrow and the peripheral blood show overcrowding with erythroid elements which become progressively immature. This neoplastic disorder affecting erythropoietic tissue is analogous in its cellular specificity to involvement of myeloid cells in leukemia. Cases have been reported in patients in younger age groups[41,61] as well as in adults. In contrast to hemolytic anemia which it resembles, the reticulocyte count is low and remains so. The disease occurs as a short and acute form of several months duration or a chronic and prolonged form lasting up to two years. Transfusions, splenectomy and steroids have been employed in treatment but the disease is ultimately fatal with hemorrhagic manifestations.

A strongly positive periodic acid-Schiff (PAS) reaction (for glycogen and mucopolysaccharides) occurs in many of the erythroblasts from the bone marrow and blood of patients with Di Guglielmo's disease.[47] A strongly positive reaction also occurs in many erythroblasts of patients with iron deficiency anemia and thalassemia major. Erythroblasts are uniformly negative in the bone marrow of normal persons and in that of patients with pernicious anemia, nutritional macrocytic anemia, aplastic anemia and polycythemia.

Erythroleukemia is a condition in which hyperplasia of erythroblastic and leukoblastic tissues are combined. The peripheral blood and bone marrow reveal an admixture of myeloblasts and immature erythroblasts.[9] According to another concept, erythremic myelosis may eventually emerge as leukemia, particularly when life is prolonged by intensive transfusion therapy.[11] In one such patient the anemia was characterized by intensive erythroblastemia, marrow erythroblastosis and a hemorrhagic state. At death the patient

quently encountered in attempts to aspirate the marrow [6] Bone marrow biopsy which is frequently necessary reveals patchy myelofibrosis hyperplastic foci of myelopoiesis and often increased numbers of megakaryocytes Roentgenographic examination reveals changes suggestive of osteosclerosis in variable parts of the skeleton such as the ribs pelvis spine and femora As the disease progresses the liver and spleen enlarge and anemia develops Evidence of increased hemolysis is indicated by fecal urobilinogen reticulocyte and red cell survival studies [3]

Although fibrosis of the bone marrow with extramedullary hematopoiesis usually occurs in adults such cases have been reported in pediatric patients The findings in the latter also consist of fibrosis of the bone marrow hepatosplenomegaly with immature granulocytic and erythrocytic elements in the peripheral blood and a predominance of megakaryocytes in the spleen and bone marrow [49]

Treatment consists of transfusions and splenectomy when blood requirements are excessive Despite the apparent need for the spleen as a source of extramedullary hematopoiesis splenectomy has often been effective when the hemolytic component accompanies its enlargement Preliminary treatment with ACTH or cortisone has been employed to determine whether it will stimulate red cell production decrease the rate of hemolysis or both Splenectomy is not undertaken until full evaluation is made especially for the presence of islands of red cells and megakaryocytes in the bone marrow and possibly in the liver The prognosis is interrelated with the underlying disease

Osteopetrosis (Marble Bone Disease, Albers Schonberg Disease) Osteopetrosis is a rare disease characterized by increased thickening and density of the cortical and spongy portions of the entire osseous system [38] The onset is usually in fetal life less often in later childhood The bones are rigid and brittle and there is a tendency for spontaneous fractures and slipping of the epiphyses to occur The individual bones including those of the skull appear opaque heavy and lacking in finer structure and in the roentgentogram normal markings are obliterated Hydrocephalus is not uncommon in infancy The disease follows a strong familial and hereditary pattern [40] It has been attributed to a failure of resorption of the calcified cartilaginous matrix during endochondral bone formation or to the influence of an unknown agent which damages the bone forming blastema [67]

The clinical picture includes anemia splenomegaly enlargement of the liver and body deformities Anemia is common but not invariable and is associated with a leukoerythroblastic response Normoblasts and myeloid cells appear in the peripheral blood in some patients resembling myeloid leukemia Thrombocytopenia may be marked and associated with massive hemorrhage No treatment is of benefit in this disease Transfusions are given as required

Extramedullary Megakaryocytosis and Acute Megakaryocytic Leukemia Hyperplasia of the bone marrow with respect to myeloid erythroid and megakaryocytic cells has been described in which the megakaryocytes predominate [13] The term megakaryocytosis in contrast to the term leukocytosis applies to an increased number of megakaryocytes in any tissue intramedullary or extramedullary usually the latter Megakaryocytes appear with an aleukemic or subleukemic blood picture often with chronic myelogenous leukemia with

Symptoms include loss of weight and appetite weakness fever cough, and dyspnea In a majority of patients the disease progresses through a latent period which is often protracted and during which nodes enlarge without symptoms This period is followed by one of further progressions and generalization during which symptoms of varying severity appear with cachexia as a terminal symptom[31] Fatigue anorexia, loss of weight, and intermittent fever (Pels-Ebstein) herald or accompany the disease The spleen is enlarged in about 60 to 70 per cent of patients with advanced disease[2]

The causative agent is unknown although viral and bacterial, especially tuberculous etiologies have been suggested but not fully established. Inflammatory and neoplastic theories have also been advanced but unproved The disease affects males more often than females with a predominance in the ratio of 3 to 1. It is most common in adults between 20 and 40 years of age and comparatively rare in children, in whom it occurs between 4 and 12 years of age and even earlier[19]

The nodes involved in order of prevalence are the cervical, axillary inguinal mediastinal, and mesenteric nodes The cervical nodes are involved in 50 to 75 per cent of all patients The nodes are usually discrete and the capsule is rarely infiltrated. The essential histologic features of Hodgkin s disease are the Reed Sternberg cells pleomorphism of the cellular tissue the presence of eosinophils and fibrosis[1] The pathologic changes vary from patient to patient and in different areas in the same patient The diagnosis is based on the distinctive histopathologic alteration in an involved area The microscopic picture is characterized by a proliferation of the large reticuloendothelial cells replacing the lymphoid tissue and the presence of giant cells derived from the reticulum cells The latter are the nucleated and multinucleated Reed Sternberg cells the pathognomonic cell and essential diagnostic feature of Hodgkin s disease The large nucleus of the Reed Sternberg cell has a well-defined membrane with many folds and outcroppings[46] Nucleoli are especially prominent The diagnostic lesions consist of eosinophils lymphocytes endothelial cells Reed Sternberg cells myelocytes and megakaryocytes Eosinophils may be especially prominent.

In the later stages the usual structure of the lymph nodes disappears and is gradually replaced by fibrous tissue From the initial manifestation of Hodgkin s disease as a painless swelling of one or more groups of superficial nodes the disease progresses Mechanical pressure of the enlarging nodes produces local clinical manifestations such as dyspnea from tracheal and mediastinal involvement. The disease may become actively invasive however extending beyond the confines of the lymph nodes to involve the spleen liver bones lungs and, to a lesser extent, other organs Radiation results in increased fibrosis and matting together of the nodes The disease is uniformly fatal within a period of a few months to several years.

Lymphosarcoma The term lymphosarcoma has been used to include all primary malignant tumors of lymphoid origin except Hodgkin s disease and has been subdivided into giant follicular lymphosarcoma, reticulum cell sarcoma, and lymphosarcoma.[40] In its restricted terminology the predominant cell in lymphosarcoma corresponds to the small mature lymphocyte so that the condition

was found to have changes characteristic of acute granulocytic leukemia[11]

Chloroma and Chloroleukemia In rare cases of acute leukemia in children and young adults there are coexisting localized tumor masses found in close relationship to the periosteum of the bones of the face ribs sternum or vertebrae and less commonly in the viscera These tumors are referred to as chloroma because of a pale greenish color on the cut surface which fades rapidly on exposure to light and air The nature of the pigment causing the green color is still controversial with theories involving white blood cell and hemoglobin derivatives These tumors consist of primitive white blood cells usually myeloblasts less commonly myelocytes and monocytes Clinically the classical case of chloroma is characterized by an orbital tumor frequently causing exophthalmos and proptosis lymphadenopathy and a rapidly progressing anemia associated with acute myelogenous and monocytic leukemia [3] A congenital case has been reported in a 13 day old infant[4]

Neoplasms of Lymphoid Tissue (Malignant Lymphomas) The malignant lymphomas include Hodgkins disease and lymphosarcoma In this classification are included diverse conditions of unknown etiology arising from lymphoid tissues The cellular constituents found in the lymph nodes and lymph follicles form the basis of specific conditions—namely lymphoblast lymphocyte reticulum cell and plasma cell Single cells alone may be involved or the histologic patterns may be more complex with the presence of nodules or granulomatous lesions of several cell types Exact diagnosis depends upon the identification of distinctive cells or a group of cells It is often impossible to distinguish between these disorders without access to complete clinical data multiple laboratory aids such as roentgenograms bone and lymph node biopsy bone marrow aspiration and blood studies Even with these facilities diagnosis is often difficult and depends upon clinical and laboratory changes during the course of the disease Transitions are known to occur from one histologic type of neoplasm to another and several different microscopic patterns are often present in the same patient and same lymph nodes[10]

Hodgkins Disease Hodgkins disease is a fatal disease characterized by a painless and progressive enlargement of the superficial and deep lymph nodes and often of other lymphoid structures of the body and of the spleen It is protean in its manifestations and during its course can resemble both tumor and infections[43] As in patients with lymphosarcoma the first evidence of Hodgkins disease may be detected in the form of a mediastinal mass in the chest observed by roentgenographic examination[9] However in contrast to lymphosarcoma in which symmetrical areas are attacked in the early stages of Hodgkins disease the pathology is usually unilateral

Hodgkins disease is classified as paragranuloma granuloma or sarcoma The paragranuloma involves chiefly lymph nodes in which the normal architecture may or may not be altered The granuloma type represents the classic form of the disease in which there is a gradual loss of normal architecture of the lymph nodes and replacement by pleomorphic cellular tissue Hodgkins sarcoma represents the most invasive and destructive form of the disease in which cells larger than lymphocytes predominate normal architecture of the nodes is destroyed and necrosis is present[15]

lymphosarcoma cell[30] is the nucleolus which stands out as a sky blue round area surrounded by a deep blue black rim of chromatin In the course of lymphosarcoma the bone marrow may become heavily infiltrated with lymphosarcoma cells without their appearance in the blood stream It may be that death ensues before the peripheral blood is invaded

We have seen a patient in whom this process appeared to have been reversed In an 11 year old child with anemia thrombocytopenia and splenomegaly the bone marrow and to a lesser extent the peripheral blood were heavily infiltrated with blast cells On the basis of a diagnosis of lymphoblastic leukemia steroids were administered and a complete remission was obtained which persisted for fifteen months At this time the onset of abdominal pain led to the discovery of a retroperitoneal lymphosarcoma Radiotherapy produced a prompt resolution of the mass One month after the beginning of this treatment leukemic cells were again found in the blood and bone marrow The initial leukemic cells may have represented metastasis from a quiescent focus of lymphosarcoma which later became reactivated

Massive enlargements of the mediastinum causing circulatory or respiratory distress which precede the blood picture of acute leukemia have been reported in children Whether the mass is of thymic origin exclusively or associated with mediastinal lymph nodes is not certain In these patients irradiation with the roentgen ray produces marked relief from the shortness of breath The mediastinal tumor disappears completely and permanently within a few days although patients have been known to succumb later to acute leukemia [7] Roentgen therapy as a factor in precipitating the leukemic phase may have been overemphasized however [4]

FOLLICULAR LYMPHOSARCOMA (GIANT FOLLICULAR LYMPHOBLASTOMA BRILL-SYMMERS DISEASE) This type of lymphosarcoma involves the lymph nodes spleen and other lymphoid tissues and is characterized by a prominence of the follicular structure Clinically it consists of generalized lymphadenopathy splenomegaly delayed appearance of anemia tendency toward pleural and peritoneal effusions bone involvement and unilateral exophthalmos Hyperplasia of lymph follicles and malpighian bodies with an increase in these elements numerically and in size is a striking feature As the disease progresses follicular hypertrophy and hyperplasia are replaced by lymphosarcoma lymphocytic leukemia or rarely Hodgkin's disease The disease is of long duration and is remarkably sensitive to x ray therapy more so than any other form of lymphoma This is particularly true in the earlier phase but less so in the later malignant phases

Blood Changes in the Lymphoma Group The degree of anemia and the white blood cell and platelet levels show great variation in patients with the malignant lymphomas In patients with the early stages of lymphosarcoma and in those with follicular lymphoblastoma the anemia may be absent or slight but eventually progresses In patients with Hodgkin's disease the anemia usually is normochromic and normocytic Various mechanisms may be involved in producing the anemia Prior to the advent of the Coombs test and techniques to determine red cell survival the anemia in patients with this group of diseases and in those with leukemia was ascribed to crowding out of the marrow by pathologic cells

is often termed small cell lymphosarcoma In the reticulum cell sarcoma the typical cell is the reticulum cell which is more than one and a half times the size of the mature lymphocyte Reticulum cell sarcoma mimics lymphosarcoma and is regarded as its most undifferentiated type Lymphosarcoma and reticulum cell sarcoma can be discussed together from the clinical pathologic and therapeutic standpoints although the latter is generally more highly malignant and invasive and metastasizes even more readily than the lymphosarcoma [1]

Lymphosarcoma occurs with greater frequency in adults than in children but in sufficient numbers in the latter to prompt a review of its clinical and hematologic aspects In a series of 583 children with malignant tumors treated at the Memorial Center twenty seven (5 per cent) had lymphosarcoma [1] The malignant neoplasms comprising this group are often difficult to classify but orientation is facilitated by considering the clinical features together with an interpretation of the specific cellular components In addition to lymphocytic and reticulum cell forms arising from the periphery and germinal center of the lymph follicle respectively lymphoblastic and mixed lymphocytic and reticulum cell types have been described

These tumors of the lymphosarcoma group originate within and outside the lymph nodes in the many anatomic regions in which lymphoid tissue is present Expansion of these areas by overgrowth of malignant cells results in destruction of the normal architecture of lymphoid tissue and infiltration of surrounding tissues The disease therefore may be limited initially to a single locus or to a region or may be generalized with constitutional symptoms and signs [14]

Symptoms and signs originate in the area of primary involvement or from the areas invaded by direct extension from affected lymph nodes The eyes nervous system skin thymus gastrointestinal tract and cervical mediastinal mesenteric and retroperitoneal nodes serve as primary sites The anterior mediastinum is frequently involved

In an appreciable number of patients lymphoblastic leukemia develops during the course of lymphosarcoma either following roentgen irradiation or occurring spontaneously regardless of the site involved Of a group of forty two children with small cell lymphosarcoma 9 to 21 4 per cent showed a transition into leukemia [9] To this association with a leukemia component the term leukosarcoma has been applied In a series of 113 patients with documented cases of reticulum cell sarcoma the course in six terminated in a syndrome resembling acute leukemia with a high percentage of immature cells in the blood and bone marrow [64]

The actual time relationship to the development of leukemia is not always apparent for examination of the blood smear may reveal a small percentage of abnormal cells at any stage of the disease These cells however may represent tumor cells rather than actual lymphoblasts Attempts have been made to differentiate between the typical lymphoblast of stem cell leukemia and the lymphosarcoma cell reaching the blood stream by invasion from the primary focus One of the main features of the lymphosarcoma cell is the coarsely reticular and more deeply staining chromatin as compared with the leptochromatic or more lightly staining nucleus of the lymphoblast A distinctive feature of the

lymphocytic tumor cell infiltrates may be found In patients with Hodgkin s disease myeloid and megakaryocytic hyperplasia is common In patients with this disease reticulum cells plasma cells polymorphonuclear leukocytes and lymphocytes are increased and in many eosinophils are conspicuous In rare cases cells resembling Reed Sternberg cells are found in bone marrow aspiration [3 8] These must be distinguished from the neighboring megakaryocytes which they resemble The cells appear as hypertrophied reticulum cells with one or several very large blue nucleoli in other cells a large number of nuclei are grouped together forming a giant cell The presence of giant nucleoli which stain pale to deep blue with Romanowsky stains serves as a distinguishing feature from megakaryocytes in which the nucleoli are small and inconspicuous and present only in the youngest cells [58] In a recent case in a boy 15 years of age these abnormal cells as well as a marked increase of eosinophils were found in the bone marrow The chief complaint was abdominal pain and marked loss of weight The lymph nodes were not enlarged and the spleen was only slightly increased in size

Lymphosarcoma and reticulum cell sarcoma can be identified more frequently in bone marrow specimens

Treatment The malignant lymphomas may be considered as a group with regard to the general principles of treatment Lymphosarcoma and Hodgkin s disease require special consideration since they figure more prominently in pediatric practice Surgery roentgen ray irradiation and chemotherapy together with supportive treatment (including transfusions and antibiotics) constitute the agents of direct attack upon these diseases

The choice and intensity of therapy depends upon whether the disease is strictly localized or is widespread Surgical excision has been recommended in the few patients in whom the lesion is confined to a single accessible focus and in whom complete examination including roentgenograms of the chest skeleton and gastrointestinal tract reveals no other involvement [9] Excisions are followed postoperatively by roentgen ray irradiation

Roentgen ray therapy however represents the most effective and dependable suppressive agent especially for localized lesions [14] It is often used alone initially or in conjunction with chemotherapy especially when patients are no longer responsive to roentgen irradiation alone Among the chemotherapeutic agents the polyfunctional alkylating agents nitrogen mustard (HN) and its derivatives constitute essential means of treatment especially in patients with Hodgkin s disease The use of nitrogen mustard with roentgen ray irradiation of selected areas constitutes an effective treatment especially in patients with disseminated lesions The sequence with which they are administered varies with each patient and frequently with special experience of a clinic

In lymphosarcoma in childhood roentgen ray therapy constitutes the mainstay of treatment Also useful are the antimetabolic drugs chiefly folic acid antagonists such as amethopterin (Methotrexate) (The dosage of amethopterin and other antimetabolic drugs will be found in the discussion on the treatment of leukemia Chapter 23) Nitrogen mustard and related compounds have little if any lasting benefit [50]

On the basis of immunologic studies the mechanism of hemolysis in many of these patients has now been shown not to differ significantly from that of patients with idiopathic autoimmune hemolytic anemia[51] Anemia reticulocytosis spherocytosis bilirubinemia of the direct type, and a positive Coombs test are conspicuous In a small number of patients autoimmune antibodies are not detected and the signs of hemolysis are slight or absent In the latter the anemia is normochromic there are no abnormalities in the red cell morphology and *transfusion requirements are occasionally increased*[9] In patients with either type red cell survival is decreased Steroids act favorably in patients with autoimmune hemolytic anemia but there is no response in those with the contrasting type

In another study no shortening of the life span was apparent until the hemoglobin level dropped below 7 5 gm per 100 ml and inadequate erythropoiesis was a major cause of anemia in patients with a negative Coombs test

The leukocyte count in patients with Hodgkin s disease may be normal low or moderately increased Leukopenia is uncommon however and lymphocytosis is rare[3] The white blood cell count in this disease usually ranges from 10 000 to 15 000 per cubic millimeter but may reach levels of 30 000 per cubic millimeter or more The higher counts usually accompany the late stages of the disease The differential count reveals a tendency toward polymorphonuclear predominance ranging from 85 to 90 per cent Monocytosis eosinophilia and lymphocytopenia characterize the peripheral blood of the patient with Hodgkin s disease Extreme degrees of eosinophilia are infrequent

The white blood cell count in patients with lymphosarcoma and follicular lymphoblastoma may be normal or there may be relative or absolute lymphocytosis Tumor cells of lymphosarcoma may be found in the blood smear Both lymphosarcoma and follicular lymphoma may be associated with lymphocytic leukemia during the progression of each disease Hematogones have been found " in increased numbers in the peripheral blood of patients with follicular lymphoblastoma These are cells smaller than lymphocytes with nuclei composed of dense chromatin and almost devoid of cytoplasm so that the cells give the appearance of naked nuclei An indentation or a horizontal crack through the cell may be seen Leukemoid blood pictures have been observed occasionally in all members of the lymphoma group

The platelet count in patients with Hodgkin s disease is usually normal Markedly increased numbers of platelets and bizarre forms occasionally present reflect the excessive numbers of megakaryocytes in the bone marrow The platelet count in patients with lymphomas other than Hodgkin s disease is usually normal or occasionally reduced Thrombocytopenia in patients with lymphomas including those with Hodgkin s disease may represent the effects of a pathologically involved spleen exerting a hypersplenic effect

Abnormal and primitive reticulum cells with morphologic features of Reed Sternberg cells have been described in the peripheral blood of a patient with Hodgkin s disease[3]

Bone Marrow Changes It has been stated[9] that bone marrow aspiration is of greatest diagnostic value in patients with lymphosarcoma in whom abnormal

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3 Bayrd E D Paulson G S and Hargraves M M Hodgkin s Specific Cells in Bone Marrow Aspiration a Brief Review and Report of Two Cases Blood 9 46 1954
4 Bogart F B Leukosarcoma Am J Roentg nol 55:743 1946
5 Cartwright G E Finch C A Loeb V Jr Moore C V Singer K and Dameshek W Panels in Therapy Splenectomy in Myeloid Metaplasia With Myelosclerosis Blood 10 550 1955
6 Cook J E Franklin J W Hamilton H E and Fowler W M Syndrome of Myelofibrosis A M A Arch Int Med 91 704 1953
7 Cooke J V Mediastinal Tumor in Acute Leukemia a Clinical and Roentgenologic Study Am J Dis Child 44 1153 1932
8 Cooper T and Watkins C H An Evaluation of Sternal Aspiration as an Aid in Diagnoses of the Malignant Lymphomata Blood 4 534 1949
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11 Dameshek W and Baldini M The Di Guglielmo Syndrome (editorial) Blood 13 192 1958
12 Dargeon H W Lymphosarcoma in Childhood In Levine S Z (editor) Advances in Pediatrics Chicago 1953 Year Book Publishers Inc
13 De La Fuente V Megakaryocytic Reaction Localized in the Bone Marrow Arch Int Med 78 387 1946
14 Diamond H D The Natural History and Management of Lymphosarcoma M Clin North America 40 721 1956
15 Diamond H D The Medical Management of Cancer New York 1958 Grune & Stratton Inc
16 Di Guglielmo G Les maladies erythemiques Rev hemat 1 355 1946
17 Discombe G and Nickol K Myelosis Involving the Granulocytic and Erythrocytic Systems J Clin Path 7 211 1954
18 Doan C A and Reinhart H L The Basophil Granulocyte Basophilcytosis and Myeloid Leukemia Basophil and Mixed Granule Types an Experimental Clinical and Pathological Study With the Report of a New Syndrome Am J Clin Path 11 1 1941
19 Douglas D M and Claireaux A E Hodgkin s Disease in Childhood Arch Dis Childhood 28 222 1953
20 Efrati P Klajman A and Spitz H Mast Cell Leukemia? Malignant Mastocytosis With Leukemia Like Manifestations Blood 12 869 1957
21 Ellis J M Urticaria Pigmentosa Report of Case With Autopsy Arch Path 48 426 1949
22 Emery J L Erythraemic Myelosis in a Girl Aged 13 Years J Path & Bact 63 395 1951
23 Erf L A and Herbut P A Primary and Secondary Myelofibrosis Clinical and Pathological Study of Thirteen Cases of Fibrosis of the Bone Marrow Ann Int Med 21 862 1944
24 Fanger H Cella L J Jr and Litchman H Thrombocythemia Report of Three Cases and Review of Literature New England J Med 250 456 1954
25 Freymann J G Burrell S B and Marler E A Role of Hemolysis in Anemia Secondary to Chronic Lymphocytic Leukemia and Certain Malignant Lymphomas New England J Med 259 847 1958
26 Friedman B I Will J J Freiman D G and Braunstein H Tissue Mast Cell Leukemia Blood 8 70 1958
27 Green T W Conley C L Ashburn L L and Peters H R Splenectomy for Myeloid Metaplasia of the Spleen New England J Med 248 211 1953
28 Groat W A Wyatt T C Zimmer S M and Field R F Acute Basophilic Leukemia, Am J M Sc 191:457 1936

Nitrogen mustard and its derivatives possess the ability to arrest mitosis and owe their effectiveness to their toxicity for all cells but more so to bone marrow lymphoid and reticulo endothelial tissues With these compounds improvement sets in within a few days with subsidence of fever and shrinkage of lymph nodes and spleen and persists from periods of a month to a year or longer Nausea vomiting and depression of the bone marrow with pancytopenia are likely to result from use of these compounds Leukopenia thrombocytopenia and anemia may become marked within a few days to a week following treatment and persist from three to five weeks Rigid attention therefore should be given to blood studies before and after treatment especially with regard to leukopenia and thrombocytopenia This caution is particularly applicable when successive courses of nitrogen mustard (HN_2) are contemplated In general it is inadvisable to employ this form of treatment when the white blood cell count is below 3 500 per cubic millimeter

HN (nitrogen mustard) the principal member of this group of drugs is given intravenously in a total dosage of 0 4 mg per kilogram of body weight It may be divided in a fractional dosage of 0 1 mg per kilogram in four consecutive days when there is evidence of abnormal depression of the bone marrow and widespread disease The calculated dose is injected into an intravenous infusion of physiologic saline solution or 5 per cent glucose in water

TEM (triethylene melamine) a compound with nitrogen mustard activity has the ad vantage of oral administration permitting ambulatory treatment The dosage is 0 12 mg per kilogram orally A single course consists of 10 mg divided into 2 5 mg doses daily for four days [14] The restoration to normal white blood cell and platelet counts is a prerequisite for repetition of treatment

Chlorambucil (Leukeran C B 1348) one of the newer nitrogen mustard derivatives is available in 2 and 4 mg tablets It is given orally in a dosage of 0 2 mg per kilogram of body weight With proper adjustment of dosage chlorambucil has been found as effective as other alkylating agents in the treatment of patients with Hodgkin s disease lymphosarcoma chronic lymphocytic leukemia and also chronic myelocytic leukemia [44] To achieve maximum results the drug is given to the point of early bone marrow depression Close observation of the hemoglobin level white blood cell count and platelet level is mandatory Chlorambucil is given initially in a dosage of 0 1 mg per kilogram when the bone marrow function has been previously impaired by radiation or chemotherapy

ACTH and the adrenocortical steroids known to cause lympholysis are useful when the disease is generalized and constitutional symptoms are present They also are important accessory therapeutic agents with the development of an acquired hemolytic anemia The antifols and 6 mercaptopurine have also been found useful especially in patients with lymphosarcoma when the disease has become generalized [1]

Hemolytic anemia may be so severe and splenomegaly so marked in patients with lymphosarcoma that splenectomy may be required in spite of continued transfusion and steroid administration to achieve stability of the hemoglobin level

With the combination of therapeutic agents the duration of life of patients with the lymphomas can be extended to many years Survivals of three and five years and longer have been reported during which the patient appeared in good health [?] In children however the course is shorter and the disease more aggressive than in adults Despite periods of remission recurrences are invariable and inevitably a fatal outcome ensues

REFERENCES

1 Anderson W A D Synopsis of Pathology ed 4 St Louis 1957 The C V Mosby Co
2 Asboe Hansen G and Kaalund Jorgensen O Systemic Mast Cell Diseas Involving

Skin Liver Bone Marrow and Blood Associated With Disseminated Xanthomata Acta haemat 16 273 1956
3 Bayrd E D Paulson G S and Hargraves M M Hodgkins Specific Cells in Bone Marrow Aspiration a Brief Review and Report of Two Cases Blood 9 46 1954
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6 Cook J E Franklin J W Hamilton H E and Fowler W M Syndrome of Myelofibrosis A M A Arch Int Med 91 704 1953
7 Cooke J V Mediastinal Tumor in Acute Leukemia a Clinical and Roentgenologic Study Am J Dis Child 44 1153 1932
8 Cooper T and Watkins C H An Evaluation of Sternal Aspiration as an Aid in Diagnoses of the Malignant Lymphomata Blood 4 534 1949
9 Craver L F Some Aspects of the Treatment of Hodgkins Disease Cancer 7 927 1954
10 Custer R P and Bernhard W G The Interrelationship of Hodgkins Disease and Other Lymphatic Tumors Am J M Sc 216 625 1948
11 Dameshek W and Baldini M The Di Guglielmo Syndrome (editorial) Blood 13:192 1958
12 Dargeon H W Lymphosarcoma in Childhood In Levine S Z (editor) Advances in Pediatrics Chicago 1953 Year Book Publishers Inc
13 De La Fuente V Megakaryocytic Reaction Localized in the Bone Marrow Arch Int Med 78 387 1946
14 Diamond H D The Natural History and Management of Lymphosarcoma M Clin North America 40:721 1956
15 Diamond H D The Medical Management of Cancer New York 1958 Grune & Stratton Inc
16 Di Guglielmo G Les maladies erythemiques Rev hemat 1 355 1946
17 Discombe G and Nickol K Myelosis Involving the Granulocytic and Erythrocytic Systems J Clin Path 7 211 1954
18 Doan C A and Reinhart H L The Basophil Granulocyte Basophilcytosis and Myeloid Leukemia Basophil and "Mixed Granule" Types an Experimental Clinical and Pathological Study With the Report of a New Syndrome Am J Clin Path 11 1 1941
19 Douglas D M and Claireaux A E Hodgkins Disease in Childhood Arch Dis Childhood 28 222 1953
20 Efrati P Klajman A and Spitz H Mast Cell Leukemia? Malignant Mastocytosis With Leukemia Like Manifestations Blood 12 869 1957
21 Ellis J M Urticaria Pigmentosa Report of Case With Autopsy Arch Path 48 426 1949
22 Emery J L Erythraemic Myelosis in a Girl Aged 13 Years J Path & Bact 63 395 1951
23 Erf L A and Herbut P A Primary and Secondary Myelofibrosis Clinical and Pathological Study of Thirteen Cases of Fibrosis of the Bone Marrow Ann Int Med 21 862 1944
24 Fanger H Cella L J Jr and Litchman H Thrombocythemia Report of Three Cases and Review of Literature New England J Med 250 456 1954
25 Freymann J G Burrell S B and Marler E A Role of Hemolysis in Anemia Secondary to Chronic Lymphocytic Leukemia and Certain Malignant Lymphomas New England J Med 259 847 1958
26 Friedman B I Will J J Freiman D G and Braunstein H Tissue Mast Cell Leukemia Blood 8 70 1958
27 Green T W Conley C L Ashburn L L and Peters H R Splenectomy for Myeloid Metaplasia of the Spleen New England J Med 248 211 1953
28 Groat W A Wyatt T C Zimmer S M and Field R E Acute Basophilic Leukemia Am J M Sc 191 457 1936

29 Hedenstrom G and Soderstrom M Di Guglielmo s Disease Comments on a Case of Erythroleucemic Myelosis Acta paediat 43 78 1954
30 Isaacs R Lymphosarcoma Cell Leukemia Ann Int Med 11 657 1937
31 Editorial Hodgkin s Disease J A M A 139 380 1949
32 Korst D R Clatanoff D V and Schilling R F On Myelofibrosis A M A Arch Int Med 97 169 1956
33 Levinson B Walter B A Wintrobe M M and Cartwright G E A Clinical Study of Hodgkin s Disease A M A Arch Int Med 99 519 1957
34 Lineman J W and Bethell F H Agnogenic Myeloid Metaplasia Its Natural History and Present Day Management Am J Med 22 107 1957
35 Ludman H and Spear P W Reed Sternberg Cells in the Peripheral Blood Report of a Case of Hodgkin s Disease Blood 12 189 1957
36 Mackenzie I and Stephenson A G A Case of Erythremic Myelosis (Di Guglielmo s Anemia) Blood 7:927 1952
37 McCausland D J M Hodgkin s Disease in Children Arch Dis Childhood 16 59 1941
38 McCune D J and Bradley C Osteopetrosis (Marble Bones) in an Infant Am J Dis Child 48:949 1934
39 McDonald J B and Hamrick J G Acute Megakaryocytic Leukemia Arch Int Med 81 73 1948
40 McPeak C N Osteopetrosis Report of Eight Cases Occurring in Three Generations of One Family Am J Roentgenol 36 816 1936
41 Menten M L and Gaffney P C Immature Cell Erythremia Am J Dis Child 80 982 1950
42 Meyer L M Halpern J and Ogden F N Acute Plasma Cell Leukemia Ann Int Med 22 585 1945
43 Meyer O O Treatment of Hodgkin s Disease J A M A 161:1484 1956
44 Miller D G Diamond H D and Craver L F The Clinical Use of Chlorambucil a Critical Study New England J Med 261:525 1959
45 Morrison M Congenital Leukemia With Chloromas Am J Dis Child 58 332 1939
46 Moss W T and Ackerman L V Plasma Cell Leukemia Blood 1:396 1946
46a Ozer F L Truax W E Miesch D C and Levin W C Primary Hemorrhagic Thrombocytopenia Am J Med 28 807 1960
47 Pisciotta A V Clinical and Pathologic Effects of Space Occupying Lesions of the Bone Marrow Am J Clin Path 20 915 1950
47a Quaglino D and Hayhoe F G J Periodic Acid Schiff Positivity in Erythroblasts With Special Reference to Di Guglielmo s Disease Brit J Haemat 6 26 1960
48 Rider T L Stein A A and Abbuhl J W Generalized Mast Cell Disease and Urticaria Pigmentosa Pediatrics 19:1023 1957
49 Rosenberg H S and Taylor F M The Myeloproliferative Syndrome in Children J Pediat 52 407 1958
50 Rosenberg S A Diamond H D Dargeon H W and Craver L F Lymphosarcoma in Childhood New England Med 259:505 1958
51 Rosenthal M C Pisciotta A V Komninos Z D Goldenberg H and Dameshek W The Auto immune Hemolytic Anemia of Malignant Lymphocytic Disease Blood 10 19 1955
52 Rosenthal N Dreskin O H Vural I L and Zak F G The Significance of Hematogones in Blood Bone Marrow and Lymph Node Aspiration in Giant Follicular Lymphoblastoma Acta haemat 8 368 1952
53 Ross R R Chloroma and Chloroleukemia Am J Med 18 671 1955
54 Schwarz L Extramedullary Megakaryocytosis in Chronic Myelosis Am J Clin Path 24 629 1954
55 Sharnoff J G Belsky H and Melton J Plasma Cell Leukemia or Multiple Myeloma With Osteosclerosis Am J Med 17:582 1954
56 Smetana A F and Cohen B M Mortality in Relation to Histologic Type in Hodgkin Disease Blood 11:211 1956

57 Sturgis C C Hematology ed 2 Springfield Ill 1955 Charles C Thomas Publisher
58 Varadi S Hodgkin s Disease Specific Findings in Sternal Puncture Material Brit J Haemat 1 184 1955
59 Wasserman L R Stats D Schwartz L and Fudenberg H Symptomatic and Hemopathic Hemolytic Anemia Am J Med 18 961 1955
60 Waters W J and Lacson P S Mast Cell Leukemia Presenting as Urticaria Pigmentosa Report of a Case Pediatrics 19 1033 1957
61 Wegelius R and Peltonen T Erythraemic Myelosis (Di Guglielmo) in an Infant Acta paediat 43 280 1954
62 Weisberger A S The Significance of Dry Tap" Bone Marrow Aspirations Am J M Sc 229 63 1955
63 Zawisch C Marble Bone Disease A Study of Osteogenesis Arch Path 43 55 1947
64 Zeffren J L and Ultmann J E Reticulum Cell Sarcoma Terminating in Acute Leukemia Blood 15 277 1960

CHAPTER 25

Disorders of the Spleen and the Reticuloendothelial System

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Role of the Spleen in Blood Disorders The spleen plays an essential part in the pathogenesis of so many blood disorders that a review of its structure and function serves as a frame of reference for appraising deviations from the normal

Structure of the Spleen The spleen is structurally organized to perform its main physiologic functions of blood formation sequestration and destruction of blood cells principally the erythrocytic series and protection against infection It possesses a smooth muscle capsule and trabeculae permitting its contraction a vascular system with connecting sinusoids allowing withdrawal of cells from the circulation a lymphoid system (malpighian corpuscles) corresponding to similar tissue elsewhere in the body and a rich supply of reticuloendothelial tissue Elements of the reticuloendothelial system are either scattered through the pulp or line the vascular and lymphatic sinusoids Whether free or fixed these cells exert a phagocytic action Mesenchymal cells and lymphoid tissue serve as a source of monocytes and lymphocytes The sequestration of blood into pulp spaces and venous sinuses exposes bacteria and particulate matter to phagocytosis by the reticuloendothelial tissue and at the same time promotes the stasis and destruction of red blood cells

Normal Functions of the Spleen The normal functions of the spleen include the production storage and destruction of blood and protection against infection

Blood Production The spleen is one of the principal sites of blood formation from the second to the fifth month of fetal life After passing through the hemocytoblast stage mesenchymal cells in the fifth month give rise to erythroblasts After the fifth month red cell production diminishes and is absent by the sixth month[1] Lymphocytes are produced in the spleen mainly in the white pulp (lymphatic tissue malpighian bodies) According to the unitarian theory monocytes are derived from lymphocytes upon migrating into the red pulp In stress situations such as hemorrhage hemolysis and leukoblastic infiltration in infants and children fetal blood foci are reactivated with the resumption of hematopoiesis This function by which the spleen liver and lymph nodes revert to their fetal function of hematopoiesis is known as extramedullary

hematopoiesis and applies equally to the production of red blood cells granulocytes and platelets The relationship of the spleen to blood formation is further confirmed by the observation that in adult mice recovery of blood forming tissues after total irradiation is accelerated by previously shielding the surgically exteriorized spleen with lead [3]

Splenic control of normal maturation of the red cell surface is indicated by the loss of stickiness of the reticulocytes during maturation the loss of reticulum and shrinkage of diameter and volume due to loss of water [1] This function is lost after splenectomy Evidence of splenic control over the bone marrow is also suggested by the peripheral blood changes occurring after splenectomy—namely prompt increase in the number of white blood cells platelets nucleated red cells and target cells tendency toward thinness of the red cells decreased osmotic fragility and appearance of siderocytes Heinz bodies and red cells containing nuclear fragments (Howell Jolly bodies) [68]

Blood Storage Although the spleen serves as a reservoir of red blood cells in the dog cat and horse evidence indicates that blood reservoirs of this nature do not exist in man [4] In the average sized adult the spleen holds 20 to 30 ml of red blood cells [1] During the passage through the spleen even normal red blood cells are rendered more fragile and a mild degree of spherocytosis is attained It has been estimated that of the 120 day life cycle of the human red blood cell about two days are spent in the spleen [17] However in patients *with pathologic conditions significant withdrawal of red blood cells from the* circulation may take place producing sudden severe anemia [47]

Blood Destruction Through the reticuloendothelial system the spleen removes worn out and fragmented cells and red cells sensitized by agglutination resulting in degradation of hemoglobin and formation of bile pigment That abnormally shaped red cells are trapped and destroyed by the normal spleen is illustrated by the fate of the spherocytes in patients with hereditary spherocytosis

Protection Against Infection It has been pointed out that the spleen with its abundant content of macrophages plasma cells and lymphocytes is strategically located in the direct stream of the circulating blood for effective phagocytosis and antibody production [4] The extent to which the spleen participates in antibody response is estimated by comparing the normal with the splenectomized animal and human being after the injection of microorganisms and other antigens It has been shown that in the rabbit abolition of antibody formation *by roentgen rays may be prevented by shielding the surgically mobilized* spleen [36] A comparison of the antibody response to different types of agents in the splenectomized and nonsplenectomized animals and human beings has led to divergent results [4] [64, 64, 66, 71] These differences may be due to the need for intravenous[64, 6] rather than subcutaneous[66] injections to demonstrate the depression of antibody response following splenectomy There is satisfactory evidence that filtration of organisms or other particulate material takes place from *the blood stream by the local phagocytic action of the macrophages in the* spleen Evidence for such a protective mechanism is suggested by experiments[9] in which intravenously injected bacteria cleared from the blood were *recovered* in the macrophages of the reticuloendothelial tissues of the liver and spleen

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3 Miscellaneous
  A Rupture of spleen
  B Cysts
  C Tumors (i e lymphosarcoma and follicular lymphoblastoma)
  D Selected cases of hypoplastic anemia (including pure red cell anemia)

In a few cases characteristically severe and fulminating infections have followed splenectomy in children Although the incidence is avowedly small in comparison with the ever increasing number of splenectomies close supervision of the splenectomized child is necessary for several years postoperatively until further information is obtained Such patients should receive immediate and energetic treatment in the event of sudden and severe illness [71]

DISORDERS OF THE SPLEEN

Splenomegaly

Elsewhere (Chapter 15) it was stated that splenomegaly is associated more commonly with the hemolytic than with other anemias In young children with anemia of comparable severity the spleen is more likely to be enlarged in those with severe Cooley s anemia than in those with sickle cell anemia or hereditary spherocytosis The enlarged spleen in the patient with sickle cell anemia becomes progressively smaller and more fibrotic as the patient gets older In interpreting the pathologic significance of an enlarged spleen it is important to remember that the spleen may be palpable in normal infants and children and at times does not recede in size until puberty At birth the weight of the spleen is approximately 10 gm at 1 year of age approximately 30 gm at 6 years of age about 55 mg at puberty approximately 95 gm and in the adult about 155 gm [8] It is estimated that the spleen must be enlarged two and a half to three times the normal size to be palpable As is shown in the following list splenomegaly accompanies both hematologic and nonhematologic disorders

1 Blood dyscrasias
  A Hemolytic anemias
    (1) Acquired hemolytic anemia
    (2) Hereditary spherocytosis
    (3) Severe Cooley s anemia
    (4) Sickle cell anemia
    (5) Many of the hemoglobinopathies
  B Leukemia
2 Infiltrative
  A Lipid
    (1) Gaucher s disease
    (2) Niemann Pick disease
  B No lipid
    (1) Letterer Siwe disease (nonlipid reticuloendotheliosis)
  C Amyloidosis
3 Vascular
  A Chronic congestive splenomegaly (Banti s syndrome)
  B Chronic passive congestion
4 Infectious

Trapping of organisms by removal mechanisms of the liver and spleen have been demonstrated for staphylococci and *Escherichia coli* [8, 9]

Properdin a natural serum protein which (in association with magnesium and complement) is involved in the destruction of selected bacteria and viruses has been found to be in lower concentration in splenectomized persons than in normal persons [10a]

Splenic Aspiration [14, 63] Splenic aspiration serves as an aid in hematologic examinations in patients with undiagnosed splenomegaly to determine the nature of the abnormal cell responsible for the condition It has been helpful in the diagnosis of lymphoma, myeloid metaplasia kala azar, and Gauchers disease especially when these conditions are suspected and bone marrow aspiration has been negative or inconclusive Aspiration of the spleen also permits observations on its pathophysiology in patients with the hemolytic disorders and in those with hypersplenic syndromes Cooperation of the patient is essential in carrying out this procedure because of the danger of excessive hemorrhage It is therefore contraindicated in patients with a hemorrhagic tendency

Adrenalin Test in Diagnosis of Hypersplenic Syndromes The adrenalin test has been employed to confirm the diagnosis of various hypersplenic syndromes with a depletion of one or more cells in the peripheral blood Epinephrine is said to prompt the mobilization of sequestered cells into the peripheral blood In one investigation [13] it was shown that this test provided only indirect corroborative evidence of the functional status of the blood forming tissues In splenomegalic conditions for instance there was no correlation between the degree of splenic contraction and the degree of hemapoietic response

Indications for Splenectomy Splenectomy is most successful in patients with hereditary spherocytosis and to a lesser extent in those with thrombocytopenic purpura By removing an inhibitory or destructive influence in patients with secondary hypersplenism it frequently restores the blood count to normal either in part or completely As mentioned elsewhere (Chapter 16) in patients with severe Cooley s anemia the beneficial effects of splenectomy result in the elimination of a hemolytic factor and in a material decrease in transfusion requirements With respect to the following conditions splenectomy is discussed in connection with management of the individual disorders

1 Hemolytic disorders
   A Hereditary spherocytosis
   B Acquired hemolytic anemia
   C In selected cases
      (1) Thalassemia major (Cooley s anemia)
      (2) Sickle cell anemia
2 Hypersplenic syndromes
   A Without splenomegaly
      (1) Chronic idiopathic thrombocytopenic purpura
   B With splenomegaly
      (1) Banti s syndrome
      (2) Splenic neutropenia
      (3) Splenic hematopenia
      (4) Gaucher s disease
      (5) Other lipid storage diseases

Gaucher's disease lymphosarcoma or Boeck's sarcoma Splenectomy frequently restores a normal peripheral blood picture without affecting the underlying disorder

Congenital Absence of the Spleen

Agenesis of the spleen usually occurs in combination with malformation of the heart commonly atrioventricular communis and partial transposition of the abdominal viscera[31, 4] Rarely is there an absence of an associated anomaly[34, 48] In either case a presumptive diagnosis of agenesis of the spleen can be made from the peripheral blood by the presence of normoblastemia and Howell Jolly bodies and Heinz bodies in the erythrocytes The diagnostic value of Heinz bodies (particles of denatured hemoglobin) has been emphasized by their presence in 10 per cent of the red cells in the peripheral blood of mature newborn infants with agenesis of the spleen[6]

Primary Splenic Neutropenia

Varying degrees of leukopenia and granulocytopenia accompany most conditions with splenomegaly in the form of a secondary hypersplenism Primary splenic neutropenia however consists strictly of neutropenia with normal granulocytes and platelets often a palpable spleen but without evidence of an underlying disease Primary splenic neutropenia is a rare disease in childhood and should be differentiated from chronic or periodic neutropenia The symptoms and signs in both conditions overlap There are frequent bouts of fever sore throat ulcerative lesions of the gums mouth tonsils vulva and vagina The leukocyte count varies between 1 000 and 3 000 per cubic millimeter with granulocytes varying from 0 to 20 per cent[1] In the typical patient symptoms are completely relieved by splenectomy

Felty's Syndrome

Felty's syndrome occurs in adults It consists of neutropenia, splenomegaly and chronic rheumatoid arthritis and usually responds to splenectomy[60]

Primary Splenic Panhematopenia

Although primary splenic panhematopenia occurs frequently in children with splenomegaly as a manifestation of secondary hypersplenism the primary disease is rare The case of a 14 year-old girl with the primary disease was described by Doan and Wright with complaints of weakness pallor unexplained fever skin disorders and anorexia accompanied by pancytopenia a hyperplastic marrow a slightly palpable nontender spleen and no adenopathy Splenectomy was followed by a hematologic as well as clinical recovery As contrasted with aplastic anemia in which pancytopenia also exists the bone marrow in patients with primary splenic panhematopenia is hyperplastic and each cellular element is present in normal or increased numbers

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A few of the better known disorders in infancy and childhood nonleukemic or neoplastic in origin are associated with a greatly enlarged spleen Out

- A Acute
  - (1) Septicemias
  - (2) Salmonella infections
  - (3) Brucellosis
  - (4) Infectious mononucleosis
- B Chronic
  - (1) Malaria
  - (2) Kala azar
  - (3) Trypanosomiasis and other parasitic infections
- C Subacute bacterial endocarditis
- D Tuberculosis
- E Sarcoidosis
- F Syphilis

5 Neoplasms and cysts
- A Lymphosarcoma
- B Hodgkin's disease
- C Follicular lymphoma
- D Reticulum cell sarcoma
- E Hemangioma and lymphangioma
- F Dermoids

6 Miscellaneous
- A Lupus erythematosus
- B Rheumatoid arthritis

Hypersplenism

The spleen exerts a regulatory influence on the control of blood formation and in the delivery of cellular elements from the bone marrow. This function is greatly exaggerated when the spleen becomes hyperactive, a condition which is termed hypersplenism and implies an exaggeration of inhibitory and destructive activities of the spleen. Hypersplenism represents a functional and not an anatomic change. Inherent in this concept is the reduction of one or more cellular elements in the peripheral blood with compensatory hyperplasia in the bone marrow of the corresponding cells, the presence of an enlarged spleen and the expectation that the peripheral blood picture will return to normal or nearly normal by splenectomy. The blood disturbances dependent upon hypersplenism have been attributed to either selective sequestration and increased destruction of formed cell elements in the enlarged spleen, or to an inhibitory influence upon a normal or hyperactive bone marrow by a hormonal mechanism.[18, 19] By inhibitory action it is understood that the growth and maturation of various cells are prevented, or else their delivery from the marrow to the blood is blocked. In either case, whether by increased destruction or inhibition, the removal of an abnormally functioning spleen has been shown to restore normal blood levels with varying degrees of success. Hypersplenism, therefore, is associated with neutropenia, thrombocytopenia, or anemia, either singly or in combination, by splenomegaly and a normal or hypercellular marrow.

Hypersplenism may be primary when there is no obvious cause for the depletion of blood cell types as there is in patients with splenic neutropenia, splenic panhematopenia or pancytopenia, and idiopathic thrombocytopenic purpura. It is secondary in patients with splenomegaly in combination with well defined disorders such as Banti's syndrome, Hodgkin's disease, chronic leukemia

Gaucher's disease, lymphosarcoma, or Boeck's sarcoma. Splenectomy frequently restores a normal peripheral blood picture without affecting the underlying disorder.

Congenital Absence of the Spleen

Agenesis of the spleen usually occurs in combination with malformation of the heart, commonly atrioventricular communis and partial transposition of the abdominal viscera.[11,24-44] Rarely is there an absence of an associated anomaly.[34,42,45] In either case a presumptive diagnosis of agenesis of the spleen can be made from the peripheral blood by the presence of normoblastemia and Howell-Jolly bodies and Heinz bodies in the erythrocytes. The diagnostic value of Heinz bodies (particles of denatured hemoglobin) has been emphasized by *their presence in 10 per cent of the red cells in the peripheral blood of mature* newborn infants with agenesis of the spleen.[6]

Primary Splenic Neutropenia

Varying degrees of leukopenia and granulocytopenia accompany most conditions with splenomegaly in the form of a secondary hypersplenism. Primary splenic neutropenia, however, consists strictly of neutropenia with normal granulocytes and platelets, often a palpable spleen, but without evidence of an underlying disease. Primary splenic neutropenia is a rare disease in childhood and should be differentiated from chronic or periodic neutropenia. The symptoms and signs in both conditions overlap. There are frequent bouts of fever, sore throat, ulcerative lesions of the gums, mouth, tonsils, vulva, and vagina. The leukocyte count varies between 1 000 and 3 000 per cubic millimeter with granulocytes varying from 0 to 20 per cent.[1] In the typical patient, symptoms are completely relieved by splenectomy.

Felty's Syndrome

Felty's syndrome occurs in adults. It consists of neutropenia, splenomegaly, and chronic rheumatoid arthritis and usually responds to splenectomy.[60]

Primary Splenic Panhematopenia

Although primary splenic panhematopenia occurs frequently in children with *splenomegaly as a manifestation of secondary hypersplenism, the primary disease* is rare. The case of a 14-year-old girl with the primary disease was described by Doan and Wright[22] with complaints of weakness, pallor, unexplained fever, skin disorders, and anorexia, accompanied by pancytopenia, a hyperplastic marrow, a slightly palpable nontender spleen, and no adenopathy. Splenectomy was followed by a hematologic as well as clinical recovery. As contrasted with aplastic anemia in which pancytopenia also exists, the bone marrow in patients with primary splenic panhematopenia is hyperplastic, and each cellular element is present in normal or increased numbers.

A few of the better known disorders in infancy and childhood, nonleukemic or neoplastic in origin, are associated with a greatly enlarged spleen. Out

A Acute
(1) Septicemias
(2) Salmonella infections
(3) Brucellosis
(4) Infectious mononucleosis
B Chronic
(1) Malaria
(2) Kala azar
(3) Trypanosomiasis and other parasitic infections
C Subacute bacterial endocarditis
D Tuberculosis
E Sarcoidosis
F Syphilis
5 Neoplasms and cysts
A Lymphosarcoma
B Hodgkin s disease
C Follicular lymphoma
D Reticulum cell sarcoma
E Hemangioma and lymphangioma
F Dermoids
6 Miscellaneous
A Lupus erythematosus
B Rheumatoid arthritis

Hypersplenism

The spleen exerts a regulatory influence on the control of blood formation and in the delivery of cellular elements from the bone marrow This function is greatly exaggerated when the spleen becomes hyperactive a condition which is termed hypersplenism and implies an exaggeration of inhibitory and destructive activities of the spleen Hypersplenism represents a functional and not an anatomic change Inherent in this concept is the reduction of one or more cellular elements in the peripheral blood with compensatory hyperplasia in the bone marrow of the corresponding cells the presence of an enlarged spleen and the expectation that the peripheral blood picture will return to normal or nearly normal by splenectomy The blood disturbances dependent upon hypersplenism have been attributed to either selective sequestration and increased destruction of formed cell elements in the enlarged spleen or to an inhibitory influence upon a normal or hyperactive bone marrow by a hormonal mechanism [18 19] By inhibitory action it is understood that the growth and maturation of various cells are prevented or else their delivery from the marrow to the blood is blocked In either case whether by increased destruction or inhibition the removal of an abnormally functioning spleen has been shown to restore normal blood levels with varying degrees of success Hypersplenism therefore is associated with neutropenia thrombocytopenia or anemia either singly or in combination by splenomegaly and a normal or hypercellular marrow

Hypersplenism may be primary when there is no obvious cause for the depletion of blood cell types as there is in patients with splenic neutropenia splenic panhematopenia or pancytopenia and idiopathic thrombocytopenic purpura It is *secondary* in patients with splenomegaly in combination with well defined disorders such as Banti s syndrome Hodgkin s disease chronic leukemia

biliary cirrhosis and portal hypertension in childhood It stems from mechanical obstruction of the bile ductules by inspissated secretions [1] Cirrhosis of the liver which accounts for 70 per cent of cases of portal hypertension in the adult is rare in children In children extrahepatic lesions are the more common and are due to congenital malformations such as congenital stenosis or atresia aneurysm of the splenic artery and distortions of the portal vein extending into the liver Other causes are thrombosis resulting from thrombophlebitis caused by omphalitis or generalized infection in early life involving splenic or portal veins or both cavernomatous transformation of the portal vein and compression from pancreatic fibrosis and tumors especially of the pancreas

Collateral Circulation In the patient with chronic portal hypertension a collateral circulation which tends to lower the pressure in the portal system develops between the portal and systemic veins These collaterals are located at the lower end of the esophagus and the upper end of the stomach in the *umbilicus and in the rectum Collateral vascular channels are sometimes seen* in the abdominal wall Of the greatest clinical significance are the collateral routes beneath the mucous membranes at the cardioesophageal junction which give rise to esophageal varices The latter are thin walled and are likely to rupture when exposed to trauma resulting in massive and at times fatal hemorrhage The hemorrhoidal varices represent connections between the portal system and systemic veins through the middle hemorrhoidal vessels and constitute a source of hemorrhage [8] Although it is known that bleeding from ruptured esophageal varices can occasionally occur in the absence of associated portal hypertension patients with a high portal venous pressure and massive bleeding from esophageal varices have been described in whom neither intrahepatic nor extrahepatic portal vein obstruction was found Esophageal varices have been observed also in patients with extensive intrahepatic Hodgkin s disease [39]

Pathology The long standing back pressure on the venous sinuses of the spleen eventually results in hemorrhage with fibrotic and regenerative changes within this organ resulting in a characteristic fibrotic spleen [86] The pathologic histology of the spleen has been described as consisting of periarterial hemor rhages developing into areas of periarterial fibrosis siderotic nodules and dilated venous sinuses with thickening of the reticulum of the wall giving a collagen staining reaction [49]

Clinical Features The onset is insidious usually with an unexplained en largement of the spleen The most common manifestations are fatigability pallor splenomegaly hematemesis or melena Portal hypertension should be suspected in children of any age even in the first year of life when hematemesis or melena is associated with an enlarged spleen In an infant who had had melena and hematemesis from 3 months of age splenoportography at 14 months revealed anomalies of the portal system In patients with fully developed chronic con gestive splenomegaly the liver is palpable and the spleen is massive and in over half of the patients hematemesis has occurred Less often hemorrhoids are a source of bleeding The bulk of these features are attributable to the rupture of varices in strategic locations Epistaxis and early bruising are noted in a small number of patients

standing examples are chronic congestive splenomegaly (Banti's syndrome) and Letterer Siwe disease Gaucher's disease and Niemann Pick disease The latter are diseases of the reticuloendothelial systems

Chronic Congestive Splenomegaly (Banti's Syndrome Portal Hypertension, Splenic Anemia)

Chronic congestive splenomegaly is characterized by enlargement of the spleen, progressive anemia leukopenia often thrombocytopenia gastrointestinal hemorrhage due to portal hypertension and in later stages cirrhosis of the liver and ascites

Etiology and Pathogenesis As originally described by Banti in 1894[6] the disease progresses in three stages The first is that of splenic enlargement and increasing anemia the second enlargement of the liver and jaundice and terminally cirrhosis of the liver gastrointestinal hemorrhage and ascites The sequence is initiated by a toxin elaborated by an enlarged spleen which acts locally and is also carried to the liver and other tissues and organs The causative agent being lodged primarily in the arteriole of the malpighian corpuscle produces a thickening of the surrounding reticulum while maintaining a gland like structure Banti designated this pathologic picture as "fibro adenie" It has since been shown[49] that "fibro adenie" was indeed a peripheral fibrosis representing a nonspecific manifestation of passive congestion and an end result of hemorrhage around the splenic arterioles More recently the concept of Banti's disease has been changed from a homogeneous entity originating in the spleen to one of congestion and enlargement of the spleen resulting from high portal venous pressure[80] Congestive splenomegaly has therefore replaced the term Banti's disease

The veins which form the portal system are the portal the superior and inferior mesenteric and the splenic veins and their tributaries The portal vein itself is formed by the union of the superior mesenteric and splenic veins Unlike other veins the portal vein ends like an artery breaking up into numerous small channels which ultimately terminate in capillaries in the substance of the liver The portal vein has no valves and carries about three fourths of the circulation of the liver whereas the hepatic artery carries oxygen and supplies the other fourth of the circulation Both vessels have a common outlet in the hepatic vein which empties into the inferior vena cava It is important to bear in mind that back pressure in the valveless portal system does not possess the anatomic barriers found in the peripheral veins[80]

The location of the obstruction in the portal system determines the type of portal hypertension If obstruction is within the liver it is classified as intrahepatic if outside the liver parenchyma it is classified as extrahepatic Normally the venous pressure in the portal system ranges from 140 to 220 mm of saline solution or from 60 to 140 mm of water[5] In the adult the portal pressure is normally below 225 mm of water so that readings above 250 mm can be regarded as abnormally high[30]

The commonest cause of portal obstruction is cirrhosis of the liver resulting from congenital diseases infiltrations hepatitis schistosomiasis and neoplasms Fibrocystic disease of the pancreas is one of the more important causes of

Newer techniques have led to an elucidation of the factors concerned with the pathogenesis of portal hypertension and the possible identification of the site of obstruction Appraisal of liver function manometric measurement of portal pressures and the use of portal venography contribute to the accuracy of diagnosis and facilitate surgical intervention for decompression of the portal area [67] In portal venography the portal venous system is rendered opaque by contrast medium and then visualized by serial roentgen studies At present percutaneous splenoportography is in common use and provides important data with regard to size and confirmation of the vessels and thus aids in the selection of the optimal type of shunt [69]

Splenorenal and portacaval anastomoses are the two types of shunts in common use and the choice of one or the other depends largely upon the caliber of the vessels available for the procedure Splenectomy might be considered preferable in children with moderate or marked enlargement of the spleen especially when pancytopenia exists Although blood values would be restored to normal or nearly normal it is now realized that once the spleen is removed the splenic vein is no longer available for anastomosis should it later become *necessary Splenectomy without a venovenous shunt is indicated only in patients* with lesions obstructing the splenic hilum [6] The prognosis is best in those children with a normal liver and with obstruction located in the splenic vein

Splenectomy with a splenorenal shunt is usually recommended in patients with marked splenomegaly hypersplenism large caliber splenic veins or an obliterated portal vein resulting from cavernomatous transformation or aplasia of the portal vein A portacaval shunt is advocated with small caliber splenic veins or cirrhosis of the liver Because of the progressive nature of the disease and the likelihood that a fair percentage of splenorenal shunts will tend to close spontaneously there has been a tendency to decompress the portal system by an immediate portacaval shunt especially in the small child Should the latter procedure eventually prove ineffective the now larger splenic vein will be available for anastomosis It is of interest that a salutory effect on the hematologic features of hypersplenism has been observed after a portacaval shunt with a reversal of the pancytopenia and without splenectomy In general it is practically impossible to establish a good shunt in a child under 2½ to 3 years of age

Anemia due to hemorrhage responds to iron therapy Blood transfusions are given for values of 8 gm per 100 ml or less but the hemoglobin concentration need not be elevated to maximal levels Esophageal bleeding remains a constant source of concern and methods for its control by tamponade with esophageal balloons have proved of questionable value

DISEASES OF THE RETICULOENDOTHELIAL SYSTEM

The reticuloendothelioses constitute a group of disorders of unknown etiology having in common hyperplasia of cellular elements of the reticuloendothelial system Reticulum cells and histiocytes undergo proliferation *principally in the* spleen liver bone marrow lymph nodes and to some extent in other tissues and organs These disorders have been subject to varied classifications but

Laboratory Data Normochromic and hypochromic anemia and leukopenia with or without thrombocytopenia are usually noted. With increasing size of the spleen marked reduction in the number of leukocytes occurs ranging usually between 1 500 and 4 000 white blood cells per cubic millimeter with a predominance of lymphocytes. Leukocytosis accompanies severe hemorrhage and when blood loss is repeated a hypochromic microcytic anemia results. In the presence of portal cirrhosis the red blood cell survival may be shortened and indirect serum bilirubin may be elevated.[4] The mechanism postulated for hemolysis is a high portal venous pressure and probably a major degree of congestive splenomegaly with hemostasis. Macrocytosis[55] and an increase in the mean corpuscular hemoglobin value have been noted in patients with long standing disease and cirrhosis of the liver.[61] In patients with marked thrombocytopenia prolonged bleeding time and occasionally increased bruising follows but this association is inconstant. The coagulation time is usually normal. The bone marrow reveals either a normal pattern despite leukopenia and anemia or a hyperplasia involving megakaryocytes and myeloid and erythroid elements.

Diagnosis The combination of massive enlargement of the spleen a palpable liver and pancytopenia often presents a difficult diagnostic problem. A history of hematemesis or melena is of course highly suggestive of portal hypersplenism. After barium is swallowed esophageal varices are visualized in only about 40 per cent of patients.[9] In patients suspected to have chronic congestive splenomegaly the use of percutaneous splenoportal venography has been helpful in diagnosis. Before a definite diagnosis can be made other conditions associated with splenomegaly leukopenia and pancytopenia must be eliminated. These include Gaucher's disease Niemann Pick disease nonlipid reticuloendotheliosis (Letterer Siwe disease) and infiltrations of the bone marrow with leukemic or neoplastic cells.

Course and Prognosis The course depends upon the degree and site of obstruction and the effectiveness of a remedial operation in relieving the excessive portal venous pressure and retarding liver damage. The control of gastrointestinal hemorrhage from ruptured varices and eradication of the obstruction are important factors in projecting the outcome. The presence of ascites and persistent anorexia are of serious prognostic import.

Treatment Adequate treatment depends upon the discovery of the site and nature of the portal obstruction. Extrahepatic portal hypertension may be differentiated from that which is secondary to liver cirrhosis[1] and is suggested by the following: a history of omphalitis or severe bacterial infection during early infancy which is followed by an uneventful course until signs of portal hypertension appear; an essentially negative and benign history prior to the onset of signs of portal hypertension such as hematemesis and/or splenomegaly; an absence of jaundice or other signs of liver disease prior to the onset of symptoms; and normal liver function tests. In contrast portal hypertension secondary to cirrhosis of the liver is suggested by positive liver function tests, evidence of a previous history of jaundice or liver enlargement, hepatomegaly and tenderness of the liver which is prominent. Hepatic enlargement, ascites and other evidences of liver failure tend to appear prior to portal hypertension

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are now separated on the basis of presence or absence of distinctive intra cellular lipids Gaucher s disease and Niemann Pick disease are the prominent members of the storage disease group and Letterer Siwe disease (nonlipid reticuloendotheliosis) Hand Schuller Christian disease and eosinophilic granuloma constitute the inflammatory group The difficulty of classification is exemplified by Hand Schuller Christian disease in which the proliferating histiocytes initially contain very little cholesterol but later accumulate enough to give the appearance of foam cells

Gaucher's Disease

Gaucher s disease is an uncommon hereditary metabolic disorder characterized by the storage of kerasin and other cerebrosides in the reticuloendothelial system *It has been most often observed in Jewish families but cases have* been described in many nationalities over the world The disease occurs as an acute infantile type and a chronic or adult type

Pathology and Pathogenesis At least three substances have been isolated from tissues containing Gaucher cells a galactosiderocerebroside (kerasin) a glucosiderocerebroside and a water soluble glycolipid polycerebroside [6] The pathogenesis of the disease has not been fully established It has been ascribed to a primary disturbance of intermediary lipid metabolism causing an elevation of cerebrosides in the serum with later storage in reticuloendothelial cells or to a primary disturbance in the reticulum cells causing increased synthesis and storage of cerebrosides [4] Postmortem examination reveals Gaucher cells *in the spleen liver lymph nodes bone marrow lungs and other organs* The disease is diagnosed by the demonstration of these cells in areas where they proliferate the most accessible of which is the bone marrow Splenic puncture also serves as a useful means of demonstrating the source of Gaucher cells These cells are large and distinctive 20 to 80 microns in diameter round or oval and possess one or more small dense nuclei eccentrically located The cytoplasm has an opaque wrinkled tissue paper appearance due to the presence of fine wavy fibrils running parallel to the long axis of the cell *The cytoplasm* occupies the major part of the Gaucher cell and stains slightly gray or bluish

Clinical Features The clinical features of the acute infantile and chronic forms of Gaucher s disease are discussed separately

Acute Infantile Form The infant may appear normal at birth and for the first weeks and months of life but soon undergoes mental and physical retardation and deterioration Splenomegaly followed by enlargement of the liver contribute to prominence of the abdomen which is further exaggerated by wasting of the extremities Severe neurologic symptoms and signs characterize the infantile form *of the disease Generalized hypertonia opisthotonus* and rigidity dysphagia laryngeal spasm cyanosis severe cough due in part to pulmonary infiltration with Gaucher cells and fever dominate the clinical picture Death follows before the age of 2 years [1] The cerebral changes observed at post mortem examination indicate chronic disease of the ganglion cells progressing to sclerosis and complete destruction Rarely typical Gaucher cells are found in the intracerebral vascular spaces [8]

Chronic Form The chronic form has an insidious onset (starting in child hood or at any age thereafter) most commonly with splenomegaly followed soon after by liver enlargement Lymphadenopathy is not a conspicuous feature The skin reveals a yellow or patchy brown pigmentation which is particularly prominent on exposed parts of the body—face neck hands and legs Pingueculae



Fig 41 *A* Photomicrograph of nest of Gaucher cells in smear of bone marrow (×900) *B* A single Gaucher cell (×1500) from the smear shown in *A* Note crinkled fibrillar appearance of cytoplasm and relatively small slightly eccentric nucleus

of the conjunctiva are rare in children and common in adults They consist of a brownish yellow wedge shaped thickening of the subconjunctival tissue with bases situated close to the corneal margins and apices pointed to the inner and outer canthi Infiltration with Gaucher cells causes pulmonary and bone involvement Pain in the legs occasionally accompanied by swelling of adjacent

joints is due to bony infiltration by Gaucher cells The roentgenogram shows diffuse or localized destructive and productive changes often producing a characteristic deformity in the lower femora This consists of a widening of the lower halves and thinning and flaring of the cortices giving an Erlenmeyer flask appearance [6] This feature is reminiscent of the swollen appearance in similar areas in patients with severe Cooley s anemia Pathologic fractures due to marked osteoporosis and replacement by Gaucher s cells may occur

Blood The anemia is usually of a mild or moderate normochromic and normocytic type Leukopenia with relative lymphocytosis and slight to marked thrombocytopenia may be present Thrombocytopenia with hemorrhage may be sufficiently severe to require splenectomy The complete pancytopenic blood picture of hypersplenic disorders may ultimately develop Serum lipid and cholesterol levels are normal

Heredity The disease has been noted in siblings in a parent and child both with the full clinical picture of Gaucher s disease [33] and in asymptomatic parents of typically affected children [9, 3] In this respect the parent can be regarded as a carrier The mode of inheritance varies with different groups the majority of cases being caused by an autosomal recessive gene [33]

Course and Treatment There is no cure for Gaucher s disease and in children beyond infancy the disease may either progress or remain chronic The lives of those who survive to adolescence are prolonged for many years Most adults die of intercurrent diseases rather than of Gaucher s disease Splenectomy is usually effective in relieving the development of a massive spleen and in reversing the severe pancytopenia [43]

Niemann Pick Disease

Niemann Pick disease is a rare heredofamilial disease resembling in its clinical features the infantile type of Gaucher s disease

Clinical Features The onset may date from birth or may occur after the first six months of life Progressive physical and mental deterioration is accompanied by massive and equal enlargement of the liver and spleen In contrast in patients with Gaucher s disease the spleen is larger than the liver A brownish yellow pigmentation occurs especially in the parts exposed to the light In an appreciable number of patients estimated as high as 60 per cent [8] a cherry red spot appears in the macula corresponding to that seen in patients with amaurotic familial idiocy (Tay Sachs disease) Nervous system involvement is manifested by spasticity blindness and deafness the patient finally lapses into a state of apathy and idiocy With the patient profoundly emaciated death usually occurs before the third year of life Although the bone marrow is infiltrated with foam cells survival is not sufficiently prolonged for changes to be conspicuously apparent on x ray examination

Chronic forms of the disease are described extending to adolescence [16] and in cases reported in two brothers death occurred at 29 and 33 years of age respectively [74] In patients who have survived beyond infancy extensive pulmonary infiltrations are observed on roentgenographic examination In a 19 year old boy under observation from early childhood with the typical Niemann

Pick cells in the bone marrow marked hepatosplenomegaly and pulmonary infiltration normal mental and physical growth took place

Pathology and Pathogenesis *The striking features at autopsy are* involvement of the liver spleen lungs bone marrow and lymph nodes and the replacement of reticular cells and histiocytes by the foam or Niemann Pick cells The nervous system is almost invariably affected Degenerative changes take place in the ganglion cells The large neurons are distended or ballooned with loss of their usual triangular or pyramidal shape Usually there are lipid deposits in the ganglion and neuroglia cells [8] Often there is a paucity of nerve cells as if many had disintegrated [16]



Fig 4_ Photomicrograph of bone marrow smear of patient with Niemann Pick disease *Note group of typical cells—their large size relatively small round or oval nuclei and foam* droplets giving a honeycomb appearance of cytoplasm

The storage material in the foam cells which accumulates in the viscera of patients with Niemann Pick disease consists largely of phospholipids chiefly lecithin and sphingomyelin These foam cells which characterize the disease are readily available for examination by bone marrow aspiration These are large *more or less rounded occasionally oval or polyhedral cells averaging 15 to 90* microns in diameter[16] and containing one or two nuclei often eccentrically placed with loosely aranged chromatin material The abundant cytoplasm is filled with highly refractile lipid droplets giving a weblike honeycombed or foamy appearance Unlike the Gaucher's cells the foam cells of the disease are readily detected in the counting chamber and can be separated from the megakaryocytes [3] (see Fig 42)

Blood Vacuoles appear in the cytoplasm of the circulating lymphocytes and monocytes but no definite histochemical studies on such cells are known

The vacuoles are discrete unstained and round and vary in number from 1 or 2 per cent to 15 or 20 per cent [18]

In the older patient anisocytosis and poikilocytosis with many oval cells are noted A mild or moderate microcytic anemia is found White blood cells vary between slight leukocytosis and moderate leukopenia Blood cholesterol either is not increased or is slightly elevated

Heredity There is a striking predilection for Jews which is more marked than in Gauchers disease The occurrence among siblings is well known Tay Sachs disease has been noted among relatives of patients with Niemann Pick disease

Treatment There is no effective treatment Splenectomy has been carried out but except for the relief of anemia and mild evidences of hypersplenism this procedure has not altered the course of the disease [16]

Letterer Siwe Disease, Hand Schuller Christian Disease, and Eosinophilic Granuloma

There is considerable evidence that these three syndromes are members of a closely related group of disorders whose underlying pathology is an inflammatory histiocytosis Each has distinctive clinicopathologic features which are currently regarded as different expressions of the same basic disorder [3] Of these Letterer Siwe disease is acute and malignant eosinophilic granuloma is the most benign and Hand Schuller Christian disease occupies an intermediate position Overlapping occurs to the extent that some histologic changes will be found in each entity which are similar to those of the other two [5]

Letterer Siwe Disease (Nonlipid Reticuloendotheliosis)

Letterer Siwe disease is an acute nonfamilial disease appearing before the age of 2 years with a rapidly fatal course within weeks or months of the onset [69 70 81] The etiology is unknown Often the disease is ushered in as an acute infection at times there is no relation to infection Irritability malaise diarrhea and development of a skin eruption which may date from birth are early features A hemorrhagic tendency manifested by petechiae and purpura combines with a yellow scaly greasy seborrheic eruption which is accentuated over the scalp and trunk [31 40 9] A purulent otitis media hepatosplenomegaly moderately enlarged and tender lymph nodes abdominal enlargement and fever characterize the disease in the infant Bronchitis and bronchopneumonia are common Circular areas of rarefraction in the calvaria and cystlike defects in the lower ends of the femora and upper ends of the tibiae have been described [15] Pulmonary involvement due to histiocytic infiltration appears on the roentgenogram as diffuse irregularly nodular involvement Progression to multiple lung cysts have been described [38]

Pathology The spleen liver lymph nodes skin lungs and bone marrow are heavily infiltrated by sheets of histiocytes granulomatous nodules and multinucleated giant cells There is no lipid storage except in occasional patients in whom there are foam cells containing cholesterol The rapid course

of the disease precludes progression to the histologic picture of Hand Schuller Christian disease.

Diagnosis A hemorrhagic tendency, eczematoid eruption enlargement of the liver spleen and lymph nodes roentgenographic evidence of bony defects and progressive anemia in a young infant are highly suggestive of Letterer Siwe disease The characteristic eruption is not invariable in the infant nor does it appear in the older child in which case diagnosis depends upon roentgenographic findings and the pathologic evidence of a generalized hyperplasia of histiocytes Biopsy of skin or lymph nodes reveals evidence of proliferating histiocytes and occasional foam cells



Fig 43 Touch preparation from the skin lesions (×725) of a 20-month old patient with nonlipid histiocytosis (Letterer Siwe disease) The large mononuclear cells resemble those seen in affected lymph nodes (From Moore T D A Simple Technique for the Diagnosis of Non Lipid Histiocytosis Pediatrics 19 438 1957)

Bone marrow aspiration often demonstrates increased numbers of histiocytes These are irregularly round oval or polyhedral cells with protoplasmic projections containing ingested particles red blood cells and leukocytes The nucleus is round oval or kidney shaped and is usually eccentrically placed[31] It should be remembered that occasional phagocytic histiocytes of this type are seen in bone marrow smears of patients with many pathologic states Skin scrapings show cells similar to the proliferating reticulum cells in the lymph nodes spleen liver and bone marow and serve as a diagnostic aid[40]

Blood Severe and progressive anemia is a prominent feature The red cells are normochromic and normocytic and the white blood cell count ranges from leukopenic to moderate leukocytosis with lymphocytosis or a normal differential count Hemohistiocytes have been identified in the peripheral blood[50] Despite the hemorrhagic nature of the disease the platelets may be normal or only

slightly decreased in numbers Occasionally there is marked thrombocytopenia Pancytopenia and a hyperplastic bone marrow are occasionally noted The etiology of the hematologic changes is by no means clear A hemolytic mechanism has been considered on the basis of persistent reticulocytosis nucleated red cells in the peripheral smears slightly elevated serum bilirubin and erythroid hyperplasia of the bone marrow [40]

Treatment and Course Supportive therapy transfusions radiation and antibiotics[10] have been employed Improvement with ACTH and steroids[8] has been reported but this form of therapy awaits further trial The disease is usually fatal in infants The chronic case in the older patient may represent a transitional form between Letterer Siwe disease and Hand Schuller Christian disease

Hand Schuller Christian Disease

Hand Schuller Christian disease is a constitutional disorder of metabolism characterized by a classic triad consisting of defects of membranous bones exophthalmos and diabetes insipidus These features and associated phenomena result from the localized accumulation of histiocytes containing cholesterol and its esters giving the appearance of foam cells (xanthomas)

Clinical Features The onset is insidious occurring most often in children before the age of 7 years and often after an infection The disease is ushered in by varied signs and symptoms indicative of underlying accumulations of histiocytes and foam cells Ulceration of the mouth loose teeth swollen gums dwarfism yellow to brownish colored plaques and nodules of the skin and chronic otitis media with mastoid involvement are characteristic findings[13, 8] Papular eczematoid lesions which resemble the dermatitis of Letterer Siwe disease start on the scalp and spread over the face and trunk Xanthomatous lesions are uncommon When present they consist of pinpoint to pea sized nodules from the deeper layer of the skin and are covered by normal epidermis[13]

Lesions in the calvaria are part of a generalized skeletal disease and consist of numerous punched out areas of bone resulting in defects of irregular size and producing a geographic pattern The defects are filled by a yellow granulomatous tissue originating in the dura or periosteum Although the skull defects are most prominent the facial bones pelvis ribs scapula and spine may be subject to granulomatous formation giving rise to localized areas of bone destruction which can be visualized on roentgenograms[51] Pathologic fractures result from cysts in the long bones and punched out areas Diabetes insipidus is due to xanthomatous involvement of the region of the hypophysis and base of the brain Exophthalmos follows destruction of the orbit and replacement by abnormal tissues Visceral involvement leads to serious complications Brain involvement is unusual Infiltration of the lungs suggesting the picture of pneumoconiosis on the roentgenogram leads ultimately to marked fibrosis cor pulmonale and right heart failure Involvement of the liver spleen and lymph nodes is less common than involvement of other systems although it may be a predominant feature Growth and sexual development are retarded

Pathology On microscopic examination the most notable histologic feature is the presence of foam cells lodged in different tissues giving a yellow coloration

The fully developed foam cell is large, pale, round or ovoid, and 20 to 40 microns in diameter, having abundant cytoplasm and one or two small nuclei. In the fresh state it is loaded with small droplets of fatlike material.[63] Biopsies show a hyperplasia of histiocytic foam cells or an admixture with granulomatous tissue rich in eosinophils. This close pathologic relationship is confirmed by cases demonstrating the transition from eosinophilic granuloma to Hand-Schuller-Christian disease.[3, 41, 60]

Diagnosis. Although membranous bone defects, exophthalmos, and diabetes insipidus are the cardinal features of Hand-Schuller-Christian disease, they are not always present together, especially in the initial stages. In one series they were present in combination in only three of twenty-nine patients. Examination of a single biopsy specimen does not permit unequivocal classification of any given case. In such a case the diagnosis and subsequent course must be deferred until the disease becomes generalized. With an incomplete picture there may be confusion with isolated bone cysts, multiple myeloma, Ewing's sarcoma, and metastatic neuroblastoma. Usually there is no anemia; if it does occur, it is mild. A severe progressive anemia is indicative of serious involvement of blood-forming organs. Serum cholesterol is normal.

Course. The course is chronic and protracted, but spontaneous remissions occur. When the onset is early in life, the course is more likely to be severe than when it comes later. Visceral and neurologic lesions presage a fulminating course.[63] Of the series of twenty-nine cases cited previously, the mortality was 13 per cent.[5] The prognosis is always doubtful, and a true estimate still awaits the results of more extensive experience with modern therapy.

Treatment. Roentgen-ray therapy results in definite improvement, being limited to the lesion toward which it is directed. In soft tissue masses and enlarged lymph nodes a response is obtained within two to three weeks, and in bone defects a response is obtained after three to four months. It is the quickest available method for healing bone lesions and for relieving exophthalmos.[44] Steroid therapy, intravenous nitrogen mustard, and the antifols, such as amethopterin, are useful adjuvants. Polydipsia and polyuria of diabetes insipidus are easily controlled by injection or insufflation of Pitressin.[44]

Eosinophilic Granuloma

Eosinophilic granuloma, the main feature of which is the presence of single or multiple skeletal lesions,[1] is regarded as the most benign member of the reticuloendothelial group.[4] It occurs predominantly in infants, children, and young adults. Any bone in the body may be involved, but there appears to be a predilection for the bones of the skull, vertebrae, extremities, and pelvis. Visceral involvement is rare. There is no hepatosplenomegaly or lymph node enlargement. Usually there is little or no systemic evidence of illness. Symptoms, when present, are referable to the local lesion and consist of mild fever, local pains, swelling, and redness. The foci of destruction as well as demarcated areas of rarefaction are represented in the roentgenograms. Expansion of the lesions in the medullary cavity of the long bones results in erosion of the cortex and spontaneous fractures. In other instances cyst formation in the long bones

is associated with regional cortical thickening[1-] The bony defects showing a punched out appearance are similar to those of Hand Schuller Christian disease Pulmonary nodular infiltrations of eosinophilic granuloma may coexist with[84] and without[39 4] associated skeletal lesions

Histologically the lesions consist of granulomatous inflammatory collections interspersed among which are polymorphonuclear leukocytes and accumulations of eosinophilic leukocytes Multinuclear giant cells and foam cells occasionally are present The number of eosinophilic leukocytes in the peripheral blood is usually not increased Transitional forms between Hand Schuller Christian disease and eosinophilic granuloma occur The lesions respond well to surgical excision or simple curettage performed at the time of biopsy Roentgen ray therapy is also effective Spontaneous cures have been observed The prognosis is favorable but must be guarded because of the possibility of skeletal lesions appearing elsewhere

REFERENCES

1 Aballi A J and Kato R Gauchers Disease in Early Infancy Review of the Literature and a Report of a Case With Neurological Symptoms J Pediat 13 364 1938
2 Abt A I and Denenholz E J Letterer Siwe s Disease Splenohepatomegaly Associated With Wide Spread Hyperplasia of Nonlipid Storing Macrophages Discussion of the So Called Reticuloendothelioses Am J Dis Child 51 499 1936
3 Ackerman L V Surgical Pathology ed 2 St Louis 1959 The C V Mosby Co
4 Allen F A Carr M H and Klotz A P Decreased Red Blood Cell Survival Time in Patients With Portal Cirrhosis Correlation of Laboratory and Clinical Findings J A M A 164 955 1957
5 Avery M E McAfee J G and Guild H G The Course and Prognosis of Reticuloendotheliosis (Eosinophilic Granuloma Schuller Christian Disease and Letterer Siwe Disease) a Study of Forty Cases Am J Med 22 636 1957
6 Banti G La splenomegalia con cirrose del fegato Sperimentale (sez biol) 48 407 1894
7 Bass M H Solitary Eosinophilic Granuloma of Bone Am J Dis Child 61 1254 1941
8 Bass M H Saplin S O and Hodes H L Use of Cortisone and Corticotrophin (ACTH) in Treatment of Reticuloendotheliosis in Children A M A Am J Dis Child 85 393 1953
9 Bennett I L Jr and Beeson P B Bacteremia a Consideration of Some Experimental and Clinical Aspects Yale J Biol & Med 26 241 1954
10 Bierman H R Lanman J T Dod K S Kelly K H Miller E R and Shimkin M B The Ameliorative Effect of Antibiotics on Nonlipid Reticuloendotheliosis (Letterer Siwe Disease) in Identical Twins J Pediat 40 1 1952
11 Bush J A and Ainger L E Congenital Absence of Spleen With Congenital Heart Disease Pediatrics 15 93 1955
12 Caffey J Pediatric X Ray Diagnosis Chicago 1956 Year Book Publishers Inc
12a Carlisle H N and Saslaw S Properdin Levels in Splenectomized Persons Proc Soc Exper Biol & Med 102 150 1959
13 Chatterjea J B Dameshek W and Stefanini M The Adrenalin (Epinephrin) Test as Applied to Hematologic Disorders Blood 8 211 19 3
14 Chatterjea J B Mesa Arrau C and Dameshek W Splenic Puncture Brit M J 1 987 1952
15 Claireaux A E and Lewis I C Reticuloendothelial Granuloma a Review With a Report of a Case of Letterer Siwe Disease Arch Dis Childhood 24 142 1950
16 Crocker A C and Farber S Niemann Pick Disease a Review of Eighteen Patients Medicine 37:1 1958

17 Crosby W H Normal Functions of the Spleen Relative to Red Blood Cells a Review Blood 14 399 1959
18 Dameshek W Hypersplenism Bull. New York Acad. Med 31 113 1955
19 Dameshek W and Estren S Hypersplenism M Clin. North America 34 1271 1950
20 de Cruchy G C Clinical Haematology in Medical Practice Springfield Ill 1958 Charles C Thomas Publisher
21 di Sant Agnese P A and Blanc W A A Distinctive Type of Biliary Cirrhosis of the Liver Associated With Disease of the Pancreas Recognition Through Signs of Portal Hypertension, Pediatrics 18 387 1956
22 Doan C A and Wright C S Primary Congenital and Secondarily Acquired Splenic Panhematopenia, Blood 1 10 1946
23 Dunnermuth, G Reticulogranulomatose Zwei Falle von Eosinophilem Granulom mit Übergang in Hand Schuller Christiansche Krankheit Helvet paediat. acta 13 15 1958
24 Ebert R V and Stead E A., Jr Demonstration That in Normal Man No Reserves of Blood Are Mobilized by Exercise Epinephrine and Hemorrhage Am J M Sc 201 655 1941
25 Ekman C A. Portal Hypertension Diagnosis and Surgical Treatment, Acta chir scandinav (suppl 222) 1 143 1957
25a Forsythe W I M Keown E F and Neill, D W Three Cases of Niemann Pick s Disease in Children Arch. Dis Childhood 34 406 1959
26 Gasser C and Willi H Spontane Innenkoperbildung bei Milzagenesie Helvet paediat acta 7 369 1952
27 Geddes A K and Moore S Acute (Infantile) Gaucher s Disease J Pediat 43 61 1953
28 Green W T and Farber S Eosinophilic or Solitary Granuloma of Bone J Bone & Joint Surg 24 499 1942
29 Groen J The Hereditary Mechanism of Gaucher s Disease Blood 2 1238 1948
30 Gross R E The Surgery of Infancy and Childhood Its Principles and Techniques Philadelphia, 1953 W B Saunders Co
31 Harvard E Rather L J and Farber H K Non Lipoid Reticuloendotheliosis (Letterer Siwe s Disease) Pediatrics 2 474 1950
32 Hsia D Y Y and Gellis S S Portal Hypertension in Infants and Children A M.A Am. J Dis Child 90 290 1955
33 Hsia D Y Y Naylor J and Bigler J A Gaucher s Disease Report of Two Cases in Father and Son and Review of the Literature New England J Med 261 164 1959
34 Ivemark, B I Implications of Agenesis of the Spleen on the Pathogenesis of Conotruncus Anomalies in Childhood Acta paediat (suppl. 104) 44 1 1955
35 Jacobson L O Marks E K Gaston E O Simmons E L and Robson M J Modification of Radiation Bull New York Acad Med. 30 675 1954
36 Jacobson L O Robson M E Marks E K and Goldman M C The Effect of X radiation on Antibody Formation J Lab & Clin Med. 34 1612, 1949
37 Jaffee H L and Lichtenstein L Eosinophilic Granuloma of the Bone a Condition Affecting One Several or Many Bones But Apparently Limited to the Skeleton and Representing the Mildest Clinical Expression of the Peculiar Inflammatory Histiocytosis Also Underlying Letterer Siwe Disease and Schuller Christian Disease Arch Path 37 99 1944
38 Keats T E and Crane J F Cystic Changes of the Lungs in Histiocytosis A M.A Am J Dis Child 88 764 1954
39 Lackey R W Leaver F Y and Fatinacci, C J Eosinophilic Granuloma of the Lung Radiology 59 504 1952
39a Levitan R Diamond, H D and Craver L F Esophageal Varices in Hodgkin s Disease Involving the Liver Am J Med 27 137 1959
40 Lipton E L Hemolytic and Pancytopenic Syndrome Associated With Letterer Siwe Disease Pediatrics 14 533 1954

41 Love F M and Fashena G J Eosinophilic Granuloma of the Bone and Hand Schuller Christian Disease J Pediat 32 46 1948
42 Mazzitello W F Eosinophilic Granuloma of the Lung New England J Med 250 804 1954
43 Medoff A S and Bayrd E D Gaucher s Disease in 29 Cases Hematologic Complications and Effects of Splenectomy Ann Int Med 40 481 1954
44 Mermann A C and Dargeon H W The Management of Certain Nonlipid Reticulo-endotheliosis 28 Cases Treated Over a 22 Year Period Cancer 8 112 1955
45 Meyerson R M Stout R and Havens W P Production of Antibody by Splenec tomized Persons Am J M Sc 234 297 1957
46 Moore T D A Simple Technique for the Diagnosis of Nonlipid Histiocytosis Pediatrics 19 438 1957
47 Motulsky A G Casserd F Giblett E R Broun G O Jr and Finch C A Anemia and the Spleen New England J Med 259 1164 1958
47a Muir C S Splenic Agenesis and Multilobulate Spleen Arch Dis Childhood 34 431 1959
48 Murphy J W and Mitchell W A Congenital Absence of the Spleen Pediatrics 20 253 1957
49 McMichael J Pathology of Hepatolienal Fibrosis J Path & Bact 39 481 1934
50 Orchard N P Letterer Siwe s Syndrome Report of a Case With Unusual Peripheral Blood Changes Arch Dis Childhood 25 151 1950
51 Otani S and Ehrlich J C Solitary Granuloma of Bone Simulating Primary Neoplasm Am J Path 16 479 1940
52 Perla D and Marmorston J The Spleen and Resistance Baltimore 1935 Williams & Wilkins Co
53 Pick L Niemann Pick Disease and Other Forms of So Called Xanthomatosis Am J M Sc 185 601 1933
54 Polhemus D W and Schafer W B Absent Spleen Syndrome Hematologic Findings as an Aid to Diagnosis Pediatrics 24 254 1959
55 Putschar W G J and Manion W C Congenital Absence of the Spleen and Associated Anomalies Am J Clin Path 26 429 1956
56 Reich C Seife M and Kessler B J Gaucher s Disease a Review and Discussion of 20 Cases Medicine 30 1 1951
56a Robinson T W and Sturgeon P Post Splenectomy Infection in Infants and Children Pediatrics 25 941 1960
57 Rodgers C L and Jackson S H Acute Infantile Gaucher s Disease Pediatrics 7 53 1951
58 Rogers D E Studies on Bacteremia Mechanisms Relating to the Persistence of Bac teremia in Rabbits Following the Intravenous Injection of *Staphylococci* J Exper Med 103 713 1956
59 Rogers D E and Melly M A Studies on Bacteremia the Blood Stream Clearance of Escherichia Coli in Rabbits J Exper Med 105 113 1957
60 Rogers H M and Langley F H Neutropenia Associated With Splenomegaly and Atrophic Arthritis (Felty s Syndrome) Report of a Case in Which Splenectomy Was Performed Ann Int Med 32 745 1950
61 Rosenberg D H Macrocytic Anemia in Liver Disease Particularly Cirrhosis Am J M Sc 192 86 1936
62 Rousselot L M The Present State of Surgery for Portal Hypertension Am J Med 16 874 1954
63 Rowland R S Constitutional Disturbances of Lipid Metabolism and the Reticulo endothelial system Constitutional Disturbances of the Lipid Metabolism In McQuarrie Irvine and Kelley Vincent C Brennemann Practice of Pediatrics Hagerstown Md W F Prior Co Inc vol 3 chap 23 p 15
64 Rowley D A The Effect of Splenectomy on the Formation of Circulating Antibody in the Adult Male Albino Rat J Immunol 64 289 1950

65 Rowley D A The Formation of Circulating Antibody in the Splenectomized Human Being Following Intravenous Injection of Heterologous Erythrocytes J Immunol 65 515 1950

66 Saslow S Bouroncle B A Wall, R L and Doan C A Studies on the Antibody Response in Splenectomized Persons New England J Med. 261 120 1959

67 Schuckmell N Grove W J and Remenchik A P The Diagnosis of Operable Portal Obstruction in Children A M A Am J Dis Child 90 69_ 1955

68 Singer K Miller E P and Dameshek W Hematologic Changes Following Splenectomy in Man with Particular Reference to Target Cells Hemolytic Index and Lysolecithin Am J M Sc 202:171 1941

69 Siwe S A Die Reticuloendotheliose—ein neues Krankheitsbild unter den Hepatosplenomegalien Ztschr Kinderh 55 212 1933

70 Siwe S The Reticulo endotheliosis in Children In Levine S Z (editor) Advances in Pediatrics vol 4 New York 1949 Interscience Publisher Inc p 117

71 Smith C H Erlandson M E Schulman I and Stern G Hazard of Severe Infections in Splenectomized Infants and Children Am J Med 22 390 1957

72 Doan C A The Spleen and Reticuloendothelial System In Sodeman W A (editor) Pathologic Physiology Philadelphia 1956 W B Saunders Co chap 29 p 552

73 Stransky E and Conchu T L Heredity in the Infantile Type of Gaucher's Disease Ann paediat 177 319 1951

74 Thannhauser S J Lipoidoses Diseases of the Cellular Lipid Metabolism ed 2 New York 1950 Oxford University Press

75 Tisdale W A Klatskin G and Glenn W W L Portal Hypertension and Bleeding Esophageal Varices Their Occurrence in the Absence of Both Intrahepatic and Extrahepatic Obstruction of the Portal Vein New England J Med 261 209 1959

76 Uzman L L The Lipoprotein of Gaucher's Disease A M A Arch Path 51 329 1951

77 Uzman L L Polycerebrosides in Gaucher's Disease A M A Arch Path 55 181 1953

78 van Creveld S The Lipoidoses In Levine S Z (editor) Advances in Pediatrics vol 6 Chicago 1953 Year Book Publishers Inc p 190

79 Varga C Richter M N and De Sanctis A G Systemic Aleukemic Reticuloendotheliosis (Letterer Siwe s Disease) Am J Dis Child 75 376 1948

80 Wallace W S Reticuloendotheliosis Hand Schuller Christian Disease and the Bone Manifestations Am J Roentgenol 62 189 1949

81 Wallgren A Systemic Reticuloendothelial Granuloma Nonlipid Reticuloendotheliosis and Schuller Christian Disease Am J Dis Child 60 471 1940

82 Watson E H and Lowrey G H Growth and Development of Children Chicago 1951 Year Book Publishers Inc

83 Watson R J Shapiro H D Ellison R R and Lichtman H C Splenic Aspiration in Clinical and Experimental Hematology Blood 10 259 1955

84 Weinstein A Francis H C and Sprofkin B F Eosinophilic Granuloma of Bone Report of a Case With Multiple Lesions of Bone and Pulmonary Infiltration Arch Int Med 79 176 1947

85 Welch C S Portal Hypertension New England J Med 243 598 1950

86 Whipple A O Problems of Portal Hypertension in Relation to Hepatosplenopathies Ann Surg 122 449 1945

87 Wiseman B K and Doan C A Primary Splenic Neutropenia a Newly Recognized Syndrome Closely Related to Congenital Hemolytic Icterus and Essential Thrombocytopenic Purpura Ann Int Med 16 1097 1942

88 Wright D O Macrocytic Anemia in Banti s Disease Ann Int Med 8 814 1935

89 Zeid S S Felson B and Schiff L Percutaneous Splenoportal Venography With Additional Comments on Transhepatic Venography Ann Int Med 52 782 1960

CHAPTER 26

Blood Coagulation

An abnormal tendency to bleed is the most obvious indication of a disturbance of hemostasis Such an event calls for an investigation of possible defects in one or more of the components which normally maintain hemostasis A description of these factors and their interaction will be reviewed as a background to discussion of the hemorrhagic disorders

The arrest of hemorrhage is a complex process involving vascular integrity platelets and a group of specific circulating proteins which are active in blood coagulation The sequence from the initial response to skin and blood vessel injury to the formation of a clot requires the proper functioning and coordination of a series of vascular and clotting mechanisms These are so closely integrated that it has been stated that a single abnormality of one of the hemostatic mechanisms does not necessarily result in bleeding if all the others are normal[109]

NORMAL HEMOSTATIC MECHANISMS

Vascular Factors When a vessel is injured sufficiently to permit the escape of blood an immediate reflex contraction occurs In the smallest venules and capillaries hemostasis is accomplished by direct adhesion of endothelial surfaces[9] Since the media of veins contain less muscle than those of arteries vasoconstriction is less marked Venous hemostasis depends mainly on the accumulation of platelets at the edges of the vessel wall which eventually occludes the vessel In the small arteries and arterioles vasoconstriction permits adherence and subsequent clumping of platelets by contracting the smooth muscle The formation of large amorphous hyaline clumps derived from the platelets (viscous metamorphosis) seals off the injured area Contraction of the vessel is reinforced by this disintegration of platelets and by the release of a powerful vasoconstrictor serotonin (5 hydroxytryptamine)[1] [1 3]

In the small arteries the platelet mass is reinforced by fibrin resulting from blood coagulation during vasoconstriction Platelets and precipitated fibrin threads form a hemostatic plug which fills the lumen of the vessel The clot subsequently retracts and is partially digested and organized and the vessel is recanalized Hemorrhage from a larger artery is controlled with difficulty until the blood pressure drops sufficiently within the vessel for a blood clot to form

Extravascular factors such as subcutaneous tissue muscle bone and skin contribute to the arrest of the hemorrhage by presenting a firm surface so that the local accumulation of blood compresses the affected blood vessel Ascorbic acid is regarded as a necessary factor for the synthesis of intercellular cement substance in the capillaries which unites the individual endothelial cells—hence its use in ill defined bleeding syndromes due to increased vascular fragility Spontaneous bleeding occurs with loss or changes in the cement substance The continuity of capillaries is further strengthened by the adherence of platelets to this substance Vitamin P is apparently also involved in some unknown manner in maintaining capillary permeability but its administration therapeutically (rutin hesperidin and bioflavinoids) has been disappointing

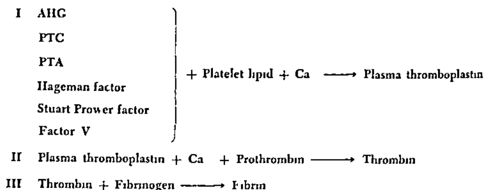
Role of Platelets Platelets normally are small granular disk shaped non nucleated bodies measuring 2 to 5 microns in diameter In patients with disease they may vary in size from fine particles to masses of agranular cytoplasm about twice the size of the normal platelet Platelets arise by budding from the cytoplasm of megakaryocytes in the bone marrow In the earlier forms of megakaryocytes *the megakaryoblast and promegakaryocyte* the cytoplasm stains deep blue is nongranular and shows no evidence of platelet formation These primitive cells are increased in numbers in patients with thrombocytopenic purpura In the mature megakaryocyte the cytoplasm is abundant and basophilic and contains numerous azurophilic granules Masses of mature platelets often adhere to the periphery of the cells The nuclei are large and are joined together in an irregularly lobulated ring

Many properties and activities associated with coagulation and hemostasis have been identified with platelet function Some of these have been mentioned *in connection with vascular factors Platelets tend to adhere to injured blood* vessels check the formation of petechiae and extravasations of blood in spontaneous hemorrhage promote clot retraction and enhance vasoconstriction It has been demonstrated that the platelet is a highly adsorptive structure and that many of the factors are merely carried on or are adherent to their surface [89] The plasma accelerator globulin (labile factor) for instance which functions in blood coagulation is adsorbed by the platelet [60] In addition to liberating a vasoconstrictor substance platelets contribute a number of factors to coagulation Platelets accelerate the conversion of prothrombin to thrombin (platelet factor 1) accelerate *the conversion of fibrinogen to fibrin (platelet factor 2) participate in* the formation of thromboplastin (platelet factor 3) and neutralize the action of heparin (platelet factor 4) An example of the relation of platelet factors to coagulation is observed in patients with severe thrombocytopenia in whom the marked reduction of platelets (less than 20 per cent of normal) retards prothrombin conversion to thrombin and is responsible for poor clot retraction [118]

The platelet factor participating in thromboplastin generation (factor 3) is a phospholipid [11] Numerous phosphatides among them brain[14] and soybean cephalins [109] have been substituted for platelets in a variety of test systems to measure thromboplastin formation

Blood Coagulation Mechanism In the classical coagulation theory of Morawitz thromboplastin originates from two sources the tissues and platelets

When tissues are injured preformed thromboplastin (thrombokinase) is liberated which in the presence of calcium converts prothrombin to thrombin Acting as an enzyme thrombin then converts fibrinogen to fibrin These studies provided a basis for subsequent investigations dealing with coagulation



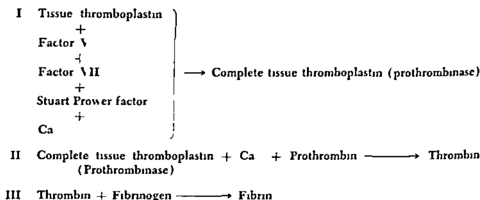


Fig 44 Schematic outline of the coagulation mechanism

It is now known that platelets contain no preformed thromboplastin but that they do interact with plasma factors to form plasma thromboplastin In the presence of calcium plasma thromboplastin converts prothrombin to thrombin without the aid of accessory factors In contrast tissue extract (incomplete thromboplastin) performs the same function but only after it has been activated by specific factors in the blood This form of thromboplastin is termed prothrombinase Plasma (intrinsic) thromboplastin evolved during the coagulation of normal blood is therefore differentiated from the extrinsic thromboplastin formed by the reaction of tissue extracts with other factors The intrinsic and extrinsic mechanisms operate concurrently the latter being more immediately mobilized for hemostasis in injured areas

Coagulation Factors Coagulation of blood free from tissue juice has been arbitrarily divided into three stages the elaboration of plasma thromboplastin

the conversion of prothrombin to thrombin and the interaction of thrombin with fibrinogen to form the fibrin clot These main stages are assisted by accelerating and inhibitory mechanisms The precise mode of action of several of the factors and their relative importance in each phase of coagulation are still being eluci dated and in many ways are still controversial

Many of the coagulation factors in plasma and serum to be discussed are described under numerous synonyms and abbreviations some of which are given in Table 17

Table 17 Coagulation Factors and Their Designations

Coagulation Factors	*Synonyms and Abbreviations*
Antihemophilic globulin	AHG AHF Thromboplastinogen Factor VIII
Plasma thromboplastin component	PTC Christmas factor Factor IX
Plasma thromboplastin antecedent	PTA
Factor V	Labile factor Plasma accelerator factor Plasma Ac globulin to serum Ac globulin Proaccelerin to accelerin
Factor VII	Stable factor Proconvertin to convertin Plasma precur or to serum prothrombin con version accelerator (SPCA) Prothrombin conversion factor

PHASE 1 The first phase of blood coagulation is concerned with the elabora tion of plasma thromboplastin free from tissue juice Contact with a foreign sur face and injury to a blood vessel initiate coagulation by the disintegration of platelets and the release of a lipoid thromboplastic factor Many cofactors react with platelets Several cofactors may be regarded as being of major importance in coagulation antihemophilic globulin (AHG)[110] plasma thromboplastin com ponent (PTC)[81, 113] plasma thromboplastin antecedent (PTA)[99, 100] factor V (labile factor) and the Stuart Prower factor[8, 36, 116, 111] Reference is also made to other participating factors factor X[4, 65a] and the Hageman factor[9, 96] Cal cium is essential These soluble plasma clotting factors with the exception of fibrinogen are present in small amounts Nevertheless for the synthesis of active

At a meeting for the standardization of nomenclature of blood clotting factors[111] it was decided that the Stuart Prower factor was similar in many respects to factor X as described by Koller[98] The committee therefore assigned the Roman X to designate the Stuart Prower factor This change does not deny the possibility of a liver factor formerly regarded as factor X which is deficient in patients with liver disease

intrinsic thromboplastin it is obligatory that they be available in adequate concentration

The importance of PTA in coagulation has been questioned by some[1] whereas others regard it as a major factor In our experience a substantial decrease of PTA results in a hemophilia like clinical picture similar in many respects to AHG and PTC deficiency

In patients with Hageman factor deficiency[85] coagulation time is prolonged but affected persons have no symptoms and have been operated upon without excessive bleeding The clot promoting effect of glass upon normal human plasma requires the presence of the Hageman factor[96] This factor a surface activation factor probably aids in the initiation of the clotting process A 9 year-old boy with a deficiency of the Hageman factor was completely free of hemorrhagic symptoms despite a prolonged clotting time[76] The content of Hageman factor may be low in many infants during the newborn period Unlike factor VII the Stuart Prower factor and plasma thromboplastin component (PTC) which do not reach normal levels until 6 weeks of age or more the concentration of Hageman factor is normal by 10 to 14 days of life[79]

The Stuart Prower factor is necessary for both the first and second phases of blood coagulation It is utilized for the production of thromboplastin as well as for the conversion of prothrombin to thrombin A deficiency of the Stuart Prower factor retards the thromboplastin generation test and prolongs the prothrombin time This factor is gradually assuming increasing clinical importance with the observation that it may be deficient in congenital and acquired liver disease and in hemorrhagic disease of the newborn infant[94]

Antihemophilic globulin is associated with the globulin of the fibrinogen fraction of the plasma protein It is labile when stored and is completely utilized in the process of clotting whereas PTC and PTA are stable when stored and are not consumed in this process These properties are of practical importance in laboratory testing and in the selection of therapeutic agents

PHASE 2 The second phase of blood coagulation is concerned with the conversion of prothrombin to thrombin In this process intrinsic thromboplastin generated in the first phase reacts with prothrombin and calcium to form thrombin Tissue juice liberated by trauma reacts with the accessory factors (factor V factor VII Stuart Prower factor and calcium) to form an extrinsic thromboplastin or prothrombinase[3b, 61, 8] The interaction of these two substances is conceived as follows active plasma thromboplastin and calcium initially convert prothrombin to thrombin slowly in small amounts At this point prothrombinase formed in the presence of calcium increases the velocity of the prothrombin conversion and is responsible for the rapid phase of thrombin formation[67] Factor V and factor VII once activated are referred to as the accelerators of prothrombin conversion They are not precursors of thrombin but are accelerators which influence the speed at which thrombin is formed in the presence of tissue extracts[1]

Although no authentic case of a hemorrhagic disease due to a deficiency of calcium has been reported calcium appears to play a role in the first two phases of coagulation This activity takes place in its diffusible ionized form The anticoagulant effect of citrate is mediated through suppression of calcium ionization whereas in the case of oxalate free calcium is precipitated

Prothrombin is a glycoprotein which is stable when stored and which is utilized in excess of 25 per cent during the coagulation process A sufficient intake of vitamin K is necessary for normal synthesis of prothrombin provided liver function is normal

Factor V deteriorates in oxalated plasma and therefore is referred to as the labile factor It is also called ac globulin and pro accelerin Factor VII is also known as stable factor pro convertin and serum prothrombin conversion accelerator (SPCA)

Factor V is consumed during clotting stable factor is not A deficiency in either factor V factor VII or Stuart Prower factor results in a hemorrhagic state due to decreased formation of prothrombinase with subsequent inadequate thrombin formation Factor V and Stuart Prower factor are also necessary for the formation of intrinsic thromboplastin in the first phase of coagulation Factor VII is not necessary for thromboplastin generation in co agulating blood in contrast to the Stuart Prower factor or for conversion of prothrombin to thrombin by the intrinsic or plasma thromboplastin Factor VII represents one of the factors that is involved in the reaction with tissue extract to form extrinsic thromboplastin which directly activates prothrombin

PHASE 3 In the final phase of coagulation fibrinogen is converted to fibrin through the action of thrombin liberated in phase 2 Fibrinogen with a molecular weight of 350 000 is an unstable soluble protein formed in the liver In the plasma fibrinogen reaches a concentration of 250 to 400 mg per 100 ml The final step clot retraction appears to be controlled by intact platelets Disorders characterized by inadequate fibrin formation include congenital afibrinogenemia and congenital and acquired hypofibrinogenemia

Dynamics of Coagulation Blood clotting should be thought of as a dynamic process moving in the direction of fibrin formation It should be emphasized that the sequence of events outlined here in three distinct phases is based upon in vitro studies They serve a convenient and useful purpose in the clinical and laboratory approach to the patient with a hemorrhagic disorder Blood coagulation in vivo probably does not occur in such sharply demarcated series of steps but in a slow initial phase and a succeeding accelerated one

As soon as a small amount of thrombin is found platelets begin to be labilized and to disintegrate This activates thromboplastin which in turn converts prothrombin to thrombin From this point the generation of thrombin takes place with increasing velocity reaching explosive force The rapid phase has been referred to as a chain reaction or an autocatalytic mechanism and assures the presence of enough thrombin[80] at the site of injury The initial slow phase parallels the period from the disintegration of platelets to the formation of small amounts of thrombin The succeeding accelerated phase extends from steps involving the rapid evolution of thrombin to the conversion of fibrinogen to fibrin Thrombin thus represents the key substance in the chain reaction of blood coagulation These changes correspond roughly to blood coagulation in a test tube in which the blood remains fluid for several minutes (slow phase) and then clots solidly in a short fraction of time (accelerated phase)

Natural Inhibitors of Coagulation With the forces favoring coagulability of the blood there are corresponding opposing physiologic inhibitors and anticoagulants which maintain its fluidity Maintaining the continuity of the vascular endothelium constitutes one of the most important factors preventing the formation of thromboplastin In vivo the formation of thrombin is counteracted by natural inhibitors such as antithrombin and heparin The latter is extracted from the liver and is also present in the granules of the mast cells The adsorption of thrombin by fibrin may also be regarded as a neutralizer of thrombin

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In patients with Hageman factor deficiency[86] coagulation time is prolonged but affected persons have no symptoms and have been operated upon without excessive bleeding The clot promoting effect of glass upon normal human plasma requires the presence of the Hageman factor[99] This factor a surface activation factor probably aids in the initiation of the clotting process A 9 year-old boy with a deficiency of the Hageman factor was completely free of hemorrhagic symptoms despite a prolonged clotting time The content of Hageman factor may be low in many infants during the newborn period Unlike factor VII the Stuart Prower factor and plasma thromboplastin component (PTC) which do not reach normal levels until 6 weeks of age or more the concentration of Hageman factor is normal by 10 to 14 days of life[70]

The Stuart Prower factor is necessary for both the first and second phases of blood coagulation It is utilized for the production of thromboplastin as well as for the conversion of prothrombin to thrombin A deficiency of the Stuart Prower factor retards the thromboplastin generation test and prolongs the prothrombin time This factor is gradually assuming increasing clinical importance with the observation that it may be deficient in congenital and acquired liver disease and in hemorrhagic disease of the newborn infant[90]

Antihemophilic globulin is associated with the globulin of the fibrinogen fraction of the plasma protein It is labile when stored and is completely utilized in the process of clotting whereas PTC and PTA are stable when stored and are not consumed in this process These properties are of practical importance in laboratory testing and in the selection of therapeutic agents

PHASE 2 The second phase of blood coagulation is concerned with the conversion of prothrombin to thrombin In this process intrinsic thromboplastin generated in the first phase reacts with prothrombin and calcium to form thrombin Tissue juice liberated by trauma reacts with the accessory factors (factor V factor VII Stuart Prower factor and calcium) to form an extrinsic thromboplastin or prothrombinase[73b 81 87] The interaction of these two substances is conceived as follows active plasma thromboplastin and calcium initially convert prothrombin to thrombin slowly in small amounts At this point prothrombinase formed in the presence of calcium increases the velocity of the prothrombin conversion and is responsible for the rapid phase of thrombin formation[87] Factor V and factor VII once activated are referred to as the accelerators of prothrombin conversion They are not precursors of thrombin but are accelerators which influence the speed at which thrombin is formed in the presence of tissue extracts[1]

Although no authentic case of a hemorrhagic disease due to a deficiency of calcium has been reported calcium appears to play a role in the first two phases of coagulation This activity takes place in its diffusible ionized form The anticoagulant effect of citrate is mediated through suppression of calcium ionization whereas in the case of oxalate free calcium is precipitated

Prothrombin is a glycoprotein which is stable when stored and which is utilized in excess of 25 per cent during the coagulation process A sufficient intake of vitamin K is necessary for normal synthesis of prothrombin provided liver function is normal

Classic hemophilia occurs sporadically in children without a previous family history in about 25 to 30 per cent of patients either because the disease has gone unrecognized or because it is the end result of a mutation with the de novo appearance of either hemophilic males or female carriers [21]

Clinical Aspects The disease is characterized by recurrent episodes of bleeding from various parts of the body occurring either spontaneously or following slight injury The tendency toward prolonged hemorrhage is observed in the first days of life either from the umbilical cord which is rare or from circumcision which is common The bleeding from the latter source may be trivial or moderate or may require transfusion because of its persistence Hemorrhage occurs almost invariably in the first year of life with the onset of walking commonly following injuries of the nose and mouth especially lacerations of the lips tongue frenulum of the upper lip and gums Excessive bleeding during inoculations or during eruption or loss of deciduous teeth suggests hemophilia

Petechiae are rare Subcutaneous and intramuscular hemorrhages are common A significant amount of blood may be lost into the large muscles such as those of the thighs and gluteal regions A person with hemophilia may bleed from the mucous membranes into the pleural or peritoneal cavities the gastrointestinal tract solid viscera or central nervous system Hematuria is common and often persistent but responds to vigorous therapy Epistaxis is uncommon in childhood Hemorrhage into the retroperitoneal region into the mesentery and into the iliopsoas may simulate acute appendicitis Abdominal pain presents complex problems in differential diagnosis More often the signs and symptoms are due to bleeding into the intestine wall and peritoneal cavity than to inflammation although mild leukocytosis can be present in either case

In addition to blood loss tissue hemorrhages may cause serious pressure effects Hematomas in strategic areas such as those in the extremities may obstruct circulation Bleeding into the tissues of the mouth neck and thorax may seriously interfere with respiration producing asphyxia

Spontaneous hemorrhages frequently are cyclic with short episodes recurring at approximately three to eight week intervals despite the constancy of the deficiency With equal deficiency of antihemophilic globulin children show variations in clinical severity

Bleeding after circumcision in one newborn infant with hemophilia and no bleeding in another with equally severe hemophilia must be due to the amount of tissue juices expressed during the procedure The cord blood from a newborn infant with classic hemophilia was found to be devoid of antihemophilic globulin [40] indicating the lack of passage of a plasma factor from the mother

Hemarthrosis The most characteristic site of hemorrhage is the joint cavities usually of the ankles knees and especially the elbows The shoulders wrists and hips may also be involved Acute hemarthroses occur suddenly with severe pain swelling heat tenderness and limitation of motion Although early hemorrhages are readily absorbed the recurrence of bleeding eventually leads to extensive damage and thickening of synovial membranes destruction of articular sur

DISORDERS DUE TO A DEFICIENCY OF FACTORS REQUIRED FOR THROMBOPLASTIN FORMATION (PHASE I OF COAGULATION)

General Consideration of the Hemophilias Until recent studies demonstrated its manifold nature, the term hemophilia was applied to a single disorder—a deficiency of the antihemophilic globulin (AHG) known to be essential for clotting and hemostasis The discovery of the additional thromboplastin precursors plasma thromboplastin component (PTC) and plasma thromboplastin antecedent (PTA) led to the realization that hemophilia was not an isolated condition but a group of entities with a similar symptomatology [illegible] The pathogenesis of each of the three members could then be attributed to a congenital inability to produce a specific thromboplastin precursor As in patients with classic hemophilia, those with other hemophilias also demonstrate a bleeding tendency from childhood and give a history of other bleeders in the family From the standpoint of management and specific therapy the separation of the hemophilias from one another has proved to be necessary

In due course methods were developed for the differentiation of each type of defect within the hemophilia group An extension of these and other techniques revealed also that many cases of clotting disorders which had heretofore been classified as hemophilia were due to deficiencies in other phases of coagulation, principally in the prothrombin complex. In all types and grades of hemophilia, the platelet count, bleeding time clot retraction prothrombin time and fibrinogen concentrations are normal.

A feature of major importance in the diagnosis of hemophilia is the variation in its severity The mild form of the disease is most deceptive since its manifestations emerge suddenly and unexpectedly after surgical procedures, especially dental extraction or tonsillectomy The degree of clinical severity in mildly affected patients is correlated to some extent with the percentage of antihemophilic globulin in the plasma.

Classic Hemophilia (Hemophilia A, AHG Deficiency)

Hereditary Aspects Classic hemophilia is a severe congenital disorder consisting of a hereditary defect in blood thromboplastin formation due to a congenital deficiency of antihemophilic globulin. The condition is inherited as a sex linked recessive mendelian trait with transmission to affected males by asymptomatic carrier females who possess a normal content of antihemophilic globulin. The daughter of an affected male is capable of transmitting the trait to half of her sons who will have the disease and half of her daughters who will be carriers Hemophilic carriers as a group show significantly lower than normal plasma antihemophilic globulin concentrations but a considerable overlap with the normal range may exist [illegible] Abnormal results with available tests for blood coagulation were obtained in a substantial number of carriers with hemophilia A (AHG deficiency) and hemophilia B (PTC deficiency) [illegible] In rare instances classic hemophilia occurs in the female as a product of the marriage between a hemophilic male and a carrier female [illegible]

given if the patient is anemic In the use of plasma a knowledge of its properties is important AHG is labile its life span in the circulation is twelve to twenty four hours and its half life is four to six hours so that 50 per cent of the initial level will remain after four hours 25 per cent after eight hours etc

Fresh plasma that is withdrawn a few hours after collection of blood frozen rapidly at —20 C and stored in a frozen state maintains its activity for variable periods of time According to one estimate fresh frozen plasma kept at —20 C loses roughly half its strength in one month after which time the remaining AHG tends to be stable[68] According to another study[9] up to half of the donor s AHG is lost during centrifugation for separation of the plasma the half life of the remainder in the wet frozen state is about sixteen weeks In contrast AHG activity in bank blood and plasma shows progressive deterioration with about 30 to 60 per cent of the initial AHG remaining after three weeks storage[68]

The plasma from freshly collected blood may be lyophilized and reconstituted with saline solution before use AHG in lyophilized plasma is also remarkably stable for as long as one year One commercial preparation of frozen lyophilized plasma (Antihemophilic Plasma*) contained AHG levels averaging 72 per cent of fresh normal plasma[92] This preparation is of value in traveling and in areas in which fresh frozen plasma is not readily available

Studies in our laboratory have confirmed the findings of Brinkhous and co workers[6] with regard to the AHG concentration required for hemostasis in patients with hemophilia They found that the minimum hemostatic level of AHG may be as low as 5 per cent of normal although some data suggested that it might be 15 to 20 per cent of normal or higher A constant level of AHG between 10 and 20 per cent is realized by administering plasma in a dose of 10 ml per kilogram of body weight initially and 5 ml per kilogram every hour[101] It has been frequently observed that active hemorrhage will persist in patients with hemophilia after inadequate amounts of plasma or blood are given although the clotting time becomes normal

The accompanying schedules provide guides for treatment of hemorrhage in patients with various clinical conditions and for preparation of patients for surgical procedures Amounts in excess of those given in the schedules may be necessary because the plasma AHG values of normal persons vary widely—in one estimate ranging from about 60 to 175 per cent of the mean[9] In treatment of patients with hemophilia the initial dose of plasma should be injected fairly rapidly (within a period of forty five minutes to one hour) in order to supply the bleeding area with adequate amounts of AHG to control hemostasis

For emergency surgery and bleeding into dangerous areas

First day—10 ml per kilogram of body weight initially
5 ml per kilogram of body weight every four hours
Second day—10 ml per kilogram of body weight every eight hours
Third day—10 ml per kilogram of body weight every twelve hours for remainder of week

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* Hyland Laboratories Los Angeles Calif

faces and erosion of bone with chronic inflammatory lesions contractures and permanent crippling

Management Treatment of the patient with severe hemophilia requires detailed attention to many aspects of the disease As an initial step it is essential to determine whether the deficiency is due to the AHG factor or PTC factor and to assay the degree of deficiency by laboratory tests The parents must be informed of the physical and emotional adjustments that will have to be made by both the patient and the family Of major importance is the recognition of the special aptitudes of the child so that necessary training will be given for a protected occupation in adult life

The individual management of the patient with hemophilia entails a knowledge of treatment of local bleeding sites and acute internal bleeding episodes of preparation before dental extraction minor operations emergency surgery and of orthopedic care

Treatment of Bleeding Treatment of bleeding consists of local measures and transfusions of whole blood or fresh frozen or lyophilized plasma

Local Measures Treatment consists of the application of a suitable coagulant and local pressure at the bleeding site For open wounds, powdered thrombin and local packing with absorbable packs such as a fibrin foam oxidized cellulose (Oxycel) or gelatin sponge (Gelfoam) saturated in a solution of topical thrombin and Adrenalin followed by firm pressure are valuable hemostatic devices When it is not possible to apply digital pressure to an accessible bleeding site a useful procedure is to spray a mixture of Adrenalin and thrombin on the local area after it has been freed of excess clot[35] Thrombin is not to be given intravenously Cauterization is always contraindicated Suturing a wound should be avoided since it may provoke more serious local bleeding and tissue necrosis Local measures are not a substitute for transfusion or replacement therapy with frozen plasma especially if the bleeding is located in a potentially dangerous area

Transfusion of Whole Blood or Plasma For the patient who has suffered extensive blood loss immediate transfusion with whole blood or packed cells is necessary Fresh whole blood is preferable if available In many patients it is unnecessary to bring the hemoglobin level to maximum concentrations levels of 10 to 11 gm per 100 ml are adequate Short of severe anemia the basic objective in treatment of the patient in the acute phase or in preparation before a surgical procedure to prevent bleeding is to inject into the circulation a sufficient amount of antihemophilic globulin to raise its concentration to a level which will assure effective hemostasis In Christmas (PTC) disease the same objective is sought

Potent concentrates of AHC from human or animal sources (bovine or pig globulin) have been successfully employed However the animal AHC material has been difficult to sterilize and because it is a foreign protein it is potentially antigenic with the hazard of anaphylactic reactions[illegible]

USE OF PLASMA—FRESH FROZEN AND LYOPHILIZED Transfusion of type specific fresh frozen plasma is the treatment of choice A whole blood transfusion is

urine output should be measured However in all patients with excessive bleeding within cavities the initial increments of the calculated dose of plasma should be given rapidly and the remaining amount given according to the clinical condition of the patient Douglas has recently recommended the use of approximately 1 liter (or 10 to 15 ml per kilogram of body weight) of fresh frozen plasma given by rapid drip within forty five minutes to one hour and repeated every six to twelve hours to maintain a level of a required 14 per cent of AHG In patients with severe bleeding 10 to 15 ml is preferably given every four to six hours

The maintenance of adequate and continuous concentrations of AHG is especially important in patients with deep tissue hemorrhages in whom important structures may be involved Such situations are encountered in the preparation for and during emergency surgery especially of an abdominal nature and in patients with central nervous system bleeding Fatalities in patients with hemophilia are often due to ill advised surgery Hemorrhage into the tissues of the tongue and throat requires active treatment with plasma and prompt pharyngeal or laryngeal intubation to prevent fatal asphyxia [9] Hemothorax usually occurs spontaneously and produces serious respiratory and cardiac embarrassment Aspiration is usually urgent and should be preceded by the rapid administration of fresh frozen plasma in adequate quantities

The use of prophylactic plasma transfusions or plasma fractions at periodic intervals carries the hazard of a refractory state due to circulating anticoagulants [9] Management of the refractory state is most unsatisfactory Withholding of blood or plasma is recommended with the hope that the anticoagulant will disappear Treatment however is to be resumed when active bleeding recurs Steroids have been given for their suppressive action on antibody formation and except for bringing about a possible improvement in patients with cutaneous hemorrhages have been largely ineffective However they are worthy of further and more extensive trial

More important in prophylaxis is the protection of the infant and young child against trauma by carefully selecting toys padding the crib removing potential sources of injury from furniture when the child begins to walk and supervising play activities With advancing age and greater understanding accidents are decreased

Treatment of Hemarthroses Hemarthroses are particularly troublesome and tend to recur Acute joint bleeding is to be treated promptly and vigorously for three to four days with plasma as suggested in the schedules for treatment Reassurance analgesics plasma therapy bed rest and elevation with slight flexion of the joint surrounded by ice packs constitute initial treatment (plastic ice bags should be kept immediately available in the refrigerator) An elastic compression bandage is useful Following arrest of hemorrhage orthopedic supervision is necessary for conserving maximum function and preventing deformities

With the control of bleeding cautious active motion within painless areas and massage are instituted until the former range of movement is obtained [34] Active motion favors absorption of residual blood and prevents contracture of capsular and pericapsular tissues [37] Instillations of hyaluronidase into the affected

Dental extraction

10 ml per kilogram of body weight one half hour before extraction
5 ml per kilogram of body weight one hour after extraction and 10 ml per kilogram of body weight every twelve hours
10 ml per kilogram of body weight daily thereafter for four days

Hemarthrosis

10 ml per kilogram of body weight once a day for at least two days
Skip third day
10 ml per kilogram of body weight on fourth day

In lacerations of the tongue lip or frenulum in an infant intensive replacement therapy will be necessary for the first two days and more moderate amounts of plasma are given for the remainder of a week to ensure the formation of a firm clot For the small infant an entire unit of fresh frozen plasma (125 ml) is injected by drip every six hours for the first forty eight hours instead of a fraction every four hours then tapering off to two units daily Sedation is necessary to prevent premature dislodging of the clot The vein is kept open by a slow drip of an electrolyte solution between plasma injections In an infant it is essential to guard against overloading the circulation with fluid by checking on urinary output

Occasionally vigorous treatment over a prolonged period with plasma will lead to progressive edema and abdominal pain without cessation of bleeding from such sites as an extracted tooth or from the kidneys We have found it expedient in this emergency to stop plasma infusions while maintaining local hemostasis After a rest period transfusions with packed cells whole blood or plasma are reinstituted depending upon the hemoglobin level

Biggs and Macfarlane[3] classified severely affected patients with spontaneous bleeding as having less than 1 per cent of AHG mildly affected patients as having more than 5 per cent and the variably affected group as having between 0 and 5 per cent Concerning treatment they state that all patients requiring transfusions had less than 30 per cent of AHG that if the AHG level can be raised to between 15 and 30 per cent postoperative bleeding will be less excessive and that if the AHG level can be raised to more than 50 per cent postoperative bleeding may be prevented Brinkhous pointed out that injected AHG is quickly redistributed between vascular and extravascular compartments Once equilibrium is established between these compartments the rate of loss of AHG from the plasma may then be an indication of its biologic half life

In the patient with hematuria rigid adherence to the schedule outlined for active bleeding is required until bleeding is completely controlled During this period the patient must be maintained on complete bed rest

Severe hematuria in a 15 year-old boy was controlled within a five day period by vigorous treatment according to the preceding schedule Each two unit dose of fresh frozen plasma (a total of 250 to 300 ml) was given within an hour in order to maintain the necessary concentration of AHG in the circulation Packed red cells were transfused when the hemoglobin concentration dropped below 9 gm per 100 ml Between treatments the vein was kept open with an electrolyte solution given by very slow drip Complete bed rest was mandatory while the urine contained significant numbers of red cells A similar successful result was obtained one year later when hematuria recurred Because of increasing weight with age the possibility of overloading the circulation by fluid must be kept in mind and

in the bones due to hemorrhages into adjacent joints[3] Hemorrhages into the spongiosa of the shafts and epiphyses produce cystic areas of rarefaction Juxta articular cysts irregular in size and distribution are an outstanding feature seen on the roentgenogram of the patient with moderate hemophilic arthropathy[6]

Marginal bony defects of the epiphyses and erosions of joint margins are noted In the joints incomplete resorption of blood and retained blood clots result in deformities disability and infrequently ankylosis Repeated hemorrhages into joint spaces may lead to accelerated maturation and hypertrophy of the adjacent epiphyses probably resulting from local hyperemia[5] (see Fig 45) With limitation of motion generalized decalcification is noted in the bones adjacent to the affected joint



Fig. 45 Roentgenograms of knees of a patient with hemophilia (AHG deficiency) *A*, Normal epiphyses of right knee *B* Note hypertrophy of epiphysis of femur and tibia of left knee due to local hyperemia from recurrent hemarthrosis

Prognosis Deaths from exsanguination following surgical procedures or severe trauma have become less common with the judicious administration of fresh plasma or blood The protection offered the infant and young child obscures the true nature of the disease which becomes apparent as the child grows older and is exposed to trauma of various kinds Hemarthrosis resulting from frequently repeated hemorrhages eventually results in crippling and disability It has been stated that the best laboratory guide to prognosis is the determination of the blood concentrations of AHG Patients with no detectable

joint have been advocated as a means of inducing rapid restoration of motion without pain in hemarthrosis [8] More extensive experience is needed to evaluate this form of treatment as well as the aspiration of joints which is the practice in some clinics

Experience in our clinic has demonstrated the value of prednisone (20 mg daily in divided doses) in reducing pain It is given for a week or ten days after joint bleeding has been controlled by plasma and in patients with milder hemarthrosis it is given without the use of plasma A combination of aspirin and small amounts of steroids has been particularly efficacious Where a joint has been repeatedly affected rehabilitation with traction casts and braces is necessary [6]

Mild Hemophilia One of the significant observations made concerning hemophilia as a result of correlating clinical manifestations with laboratory findings is the variation in its severity On the basis of assay it appears that severely affected persons have no measurable amounts of antihemophilic globulin in the plasma in the most mildly affected patients it ranges from 5 to 20 per cent and in a smaller number it is less than 5 per cent [3] In general AHG levels above 5 per cent occur *in mildly affected patients* [3] *Under strain of the hemostatic mechanism such as* occurs postoperatively occasionally a seemingly well person with an AHG value between 30 and 45 per cent may suffer from transient bleeding It has been stated that each person appears to have a constant and characteristic level of this clotting factor It should again be emphasized that patients with mild deficiencies may not manifest hemorrhage until they sustain a sizeable injury or after such common surgical procedures as tonsillectomy or dental extraction

Diagnosis An abnormal bleeding tendency particularly hemarthroses dating from infancy occurring also in other members of the family and being limited to male members with evidence of sex linked inheritance suggests hemophilia In patients with severe grades of hemophilia the marked prolongation of whole blood clotting time is a characteristic feature In patients with mild types of disease the coagulation time may be slightly prolonged or even normal since as little as 1 per cent of AHG is sufficient to produce a normal clotting time The prothrombin consumption test and the thromboplastin generation tests are more sensitive tests of an AHG deficiency with amounts below 5 per cent and 20 per cent respectively in the plasma

The thromboplastin generation test by identifying the deficient factor will separate hemophilia B due to PTC defect from classic hemophilia which it closely resembles clinically In the type of pseudohemophilia (von Willebrand s disease) in which there is a combined vascular defect and AHG deficiency epistaxis and a prolonged bleeding time characterize the former and hemarthrosis and a normal bleeding time the latter

Mixing tests provide simple diagnostic leads The three types of plasma correct unrelated hemophilias but not those with the corresponding defect

Roentgenographic Findings In the early stages of hemophilia x ray findings are nonspecific and consist of swelling and distention of the joint Skeletal lesions result from bleeding directly into the bones or from secondary changes

In still another group an abnormality of platelets is an added feature.[91a] A reduction in the plasma concentration of antihemophilic globulin was observed in the members of four families suffering from von Willebrand's disease.[91b] Such persons were clinically affected.

Plasma Thromboplastin Component Deficiency (Hemophilia B, Christmas Disease)

Plasma thromboplastin component deficiency,[6,19,103] often designated as Christmas disease,* is a hemophilia-like disease due to a deficiency of plasma thromboplastin component (PTC), one of the necessary thromboplastin precursors. It is inherited as a sex-linked recessive and therefore is confined to males and transmitted by a carrier female as is classic hemophilia. As in patients with AHG deficiency there is no family history in about 25 to 30 per cent of the patients.

Clinical and Laboratory Features. PTC deficiency constitutes about 15 per cent of all hemophilias and is clinically indistinguishable from classic hemophilia. It also exists as a mild disease. Hemarthroses are common. The clotting time is prolonged (normal in patients with the mild form of the disease), and prothrombin consumption is abnormal (due to inadequate thromboplastin formation), but the actual defect is diagnosed by the thromboplastin generation test (located in the serum component as contrasted with the plasma component in patients with classic hemophilia). In newborn infants with hemorrhagic disease and in patients with liver disease, PTC deficiency is often found in combination with prothrombin and stable factor deficiencies. An assay of the PTC factor[3] demonstrated that a 30 per cent concentration of the factor is sufficient to protect against ordinary every day stresses and as much as 60 per cent is needed to stop bleeding from major wounds. These figures correspond to 5 to 10 per cent and 30 to 40 per cent AHG, respectively, under the same conditions in patients with classic hemophilia. Of seven carrier females and one probable carrier, four had abnormally low PTC levels.

Treatment. Accuracy of diagnosis, especially differentiation from AHG deficiency, is important. The stability of PTC when stored permits the use of banked blood or plasma. Fresh plasma, therefore, is not essential as it is in treatment of hemophilia. On the other hand, because of the greater availability of fresh frozen plasma, it is usually employed. Since the critical level of PTC for effective hemostasis is similar to that of AHG in patients with hemophilia A, comparable amounts of plasma are given. The intervals between administration may be prolonged to forty-eight to seventy-two hours because of the longer survival of PTC than AHG in the blood stream. Details of management as to dental extraction, preparation for surgery, and treatment of hemarthroses are similar to those for patients with classic hemophilia. Specific inhibitors of PTC, as of AHG, have been known to develop,[4] probably as a result of repeated transfusions of whole blood and plasma.

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* After [illegible]-year-old boy named Christmas who was among the first patients in whom this condition [illegible] diagnosed.[6]

AHG are usually severely affected and those with more than 5 per cent are almost invariably mildly affected.[3]

Vascular Hemophilia (von Willebrand's Disease, Pseudohemophilia B)

Vascular hemophilia is characterized by a moderate to severe AHG deficiency combined with a capillary defect causing defective prothrombin consumption and prolonged bleeding time.[8, 33, 91, 103, 110, 114] It was originally described by von Willebrand[110] as pseudohemophilia. The main features consist of a markedly prolonged bleeding time, normal clotting time, normal platelet count, normal clot retraction, and occurrence in both males and females.

With the advent of newer techniques, investigation showed that von Willebrand's disease comprised two groups: patients with a vascular (capillary) defect plus AHG deficiency (vascular hemophilia,[104] pseudohemophilia B[107]) and patients with a vascular defect with a normal coagulation status (pseudohemophilia A). The morphologic vascular abnormalities consisted of coiled, tortuous capillaries in the nail beds and in the venules of the bulbar conjunctivae. These abnormalities were present with or without associated deficiency of antihemophilic globulin and may account for the characteristic prolonged bleeding time in the von Willebrand syndrome.

Although severe intracranial hemorrhage can occur, the most common symptom in either group of patients, regardless of associated AHG deficiency, is severe and spontaneous epistaxis. Also common to both groups is bleeding from the tongue, gums, and teeth following extraction or loss of deciduous teeth. Excessive bruising on slight trauma is also present. The outstanding laboratory finding is a prolonged bleeding time. The clotting time, prothrombin time, platelet count, and clot retractions are normal. The capillary fragility is only occasionally abnormal. The coagulation abnormality, deficiency of AHG, if severe enough, may be reflected in the impaired prothrombin consumption and defective thromboplastin generation tests. The amount of antihemophilic globulin in patients with vascular hemophilia is in excess of that found in those with classic hemophilia and corresponds to concentrations in patients with mild hemophilia (about 5 per cent of AHG).

The presence of severe epistaxis, which characterizes vascular hemophilia, separates this disease from mild hemophilia, in which the bleeding time is normal. The disease is familial, occurs in both sexes, is probably hereditary, and is transmitted as a simple dominant. Administration of fresh plasma corrects the coagulation defect and has proved of value in treatment before and after dental extraction. Splenectomy is definitely contraindicated. Vitamin P, vitamin C, rutin, and steroids have proved ineffective in controlling epistaxis and other manifestations of the abnormal vascular component.

The fact that transfusion of normal plasma[11] or a plasma fraction[84, 110] occasionally corrects the bleeding time as well as the coagulation time suggests the possibility that vascular dysfunction may result from a deficiency of a plasma vascular factor required for normal vasoconstriction which is different from AHG

fore consists of prothrombin and labile stable and Stuart Prower factors Each deficiency will be included in this discussion of phase 2 coagulation although labile and Stuart Prower factors are also involved in the first phase of coagulation with blood thromboplastin formation

Deficiencies of the factors of the prothrombin complex may be congenital in origin or may be acquired in various disease states Members of the prothrombin complex are synthesized in the liver so that deficiency of one or all of the factors is found in patients with liver disease Combined deficiencies of the four components are most commonly found in patients with parenchymal liver disease such as infectious hepatitis and cirrhosis Since prothrombin and stable factor deficiencies are usually found in combination it is rare to find a disease condition in which one occurs to the exclusion of the other Deficiency of both factors may be further associated with plasma thromboplastin component (PTC) deficiency in patients with liver disease and in newborn infants with hemorrhagic disease [4] Deficiency in the latter may be mainly due to liver immaturity

Hypoprothrombinemia (Vitamin K and Prothrombin Deficiency)

Vitamin K is a dietary principle required for the normal clotting of blood In chicks fed fat free diets Dam[3] observed the development of hemorrhages in various tissues and noted further that it was associated with a longer clotting time of the blood It was soon determined that the delay in clotting resulted from a decrease in prothrombin and that the hemorrhagic tendency resulting from this deficiency could be corrected by feeding the chicks substances containing an active principle vitamin K Prothrombin is synthesized by the liver from vitamin K and absorbed by the intestine but the mechanism by which synthesis is achieved is still unknown Deficiencies of vitamin K regardless of cause are now known to result in a depletion of both prothrombin and stable factors but not in labile factor The administration of vitamin K corrects both prothrombin and stable factor deficiencies

Hypoprothombinemia combined with stable factor deficiency occurs in various states in which vitamin K is reduced Since natural vitamin K is fat soluble bile is required for its absorption A deficiency is very likely to occur whenever bile is prevented from entering the intestinal tract as occurs in patients with biliary obstruction and in those with hepatic disease due to toxic and infectious agents since the reactions of vitamin K in prothrombin synthesis are impaired Deficiencies occur in the presence of faulty intestinal absorption such as occurs in patients with celiac disease diarrhea gastrointestinal malformation and steatorrhea Green vegetables and products of intestinal bacteria are among the richest sources of vitamin K Hypoprothrombinemia due to a deficient intake of this vitamin is rare because prothrombin is capable of being elaborated from the small amounts of this vitamin commonly found in foods and also produced by the intestinal flora In newborn infants hypoprothrombinemia occurs in the first day of life until the normal intestinal bacterial flora become established

Combined prothrombin and stable factor deficiency is also induced by dicoumarin and related drugs the predominating defect being in the stable factor

The clinical response in two children with PTC deficiency treated with small transfusions of serum (100 to 200 ml of serum previously frozen and stored at —10 C) was more prompt and prolonged than with whole blood or fresh frozen plasma.[1, 90a]

Plasma Thromboplastin Antecedent Deficiency (Hemophilia C)

Plasma thromboplastin antecedent deficiency is due to a deficiency of PTA which is required for thromboplastin formation and occurs as an inherited abnormality.[99, 100] In contrast to classic hemophilia and PTC deficiency this form of hemophilia is transmitted as a dominant with no sex linkage. Sporadic cases are observed as is true of the other hemophilias described.

Clinical and Laboratory Features The hemorrhagic tendency is usually milder than in patients with AHG or PTC deficiencies. Spontaneous hemorrhage is rare but may occur as in a recently reported patient with cerebral hemorrhage.[6] Usually bleeding is related to trauma. Because of the infrequency of spontaneous bleeding and the relative mildness of the bleeding PTA deficiency often escapes detection. As in patients with the other types of hemophilia excessive bleeding may follow minor injuries and surgical procedures usually dental extraction and tonsillectomy. Easy bruising and occasionally spontaneous epistaxis have been noted but hemarthroses are uncommon.

Usually the whole blood clotting time is normal or only slightly prolonged and except in the most mildly affected patients prothrombin consumption is abnormal. The deficiency of PTA is detected by the thromboplastin generation test (abnormality in both the serum and plasma component as contrasted with the plasma component alone in AHG deficiency and serum component alone in PTC deficiency.)

Treatment Plasma thromboplastin antecedent is very stable when stored permitting the use of banked blood or plasma. The amounts to be given are similar to those used in the treatment of AHG and PTC deficiency with intervals of twenty four to forty eight hours. This applies to treatment of the acute bleeding emergency as well as to preparation for a surgical procedure. Here too fresh frozen plasma is often given because of its greater availability.

DISORDERS DUE TO A DEFICIENCY OF FACTORS REQUIRED FOR THE CONVERSION OF PROTHROMBIN TO THROMBIN (PHASE 2 OF COAGULATION)

General Considerations Disturbances in the second stage of coagulation result in a deficient formation of thrombin. Both intrinsic (plasma) and extrinsic thromboplastin (prothrombinase) activate the conversion of prothrombin to thrombin in the presence of calcium so that both mechanisms play an important part in hemostasis. It has already been stated that the formation of extrinsic thromboplastin depends upon the interaction of tissue extract and labile (V) stable (VII) and Stuart Prower factors. The deficiency of these factors as well as of prothrombin is most conveniently determined in the laboratory in connection with the second stage of coagulation. The prothrombin complex there

and in the presence of liver disease, leukemia, malignancies, pernicious anemia and abruptio placentae. Factor V deficiency has been reported in patients with hemorrhagic scarlet fever (purpura fulminans).[60]

Stable Factor Deficiency (Factor VII Deficiency, Proconvertin Deficiency, Congenital Hypoproconvertinemia)

Isolated stable factor deficiency has been reported[10,40,50,63,64] but its incidence is rare. Affected patients give a history of excessive bleeding dating from early life.[13] Hemorrhagic phenomena are seen in patients with factor VII concentrations of approximately 10 per cent of normal or less.[7] Bruising, marked epistaxis, hematuria, hemarthrosis, melena, hematoma and often bleeding after dental extraction are common phenomena in this group. Spontaneous hemorrhages have been described in the central nervous system, especially subarachnoid bleeding. The disease is familial and is transmitted as a simple dominant. A decreased activity of stable factor may be found in relatives who show greatly decreased amounts of this factor in the blood. In one family intracranial hemorrhage was the cause of death of the father and the siblings.[5]

The one stage plasma prothrombin time of Quick is prolonged and is corrected by the addition of serum (fresh or stored) which contains stable factor but is not corrected by prothrombin free plasma (barium sulfate treated). The bleeding and whole blood clotting time may be normal or prolonged. Clotting time is normal in the presence of small amounts of stable factor but is prolonged in its complete absence. Acquired stable factor deficiency occurs in patients with many pathologic conditions, probably always with some prothrombin defect (such as are caused by dicoumarin and tromexan therapy).

Treatment consists of the transfusion of banked blood or plasma. Multiple transfusions are usually necessary. Serum, which is a rich source of factor VII, is very effective. The amounts to be given can be controlled by the reduction in prothrombin time. Vitamin K is ineffective since it will not correct a congenital defect in which the inability of the end organ, the liver, to synthesize prothrombin and stable factor exists.

Hemorrhagic Disease of the Newborn Infant (Hypoprothrombinemia in the Newborn Infant)

Hemorrhagic disease of the newborn infant is a self limited hemorrhagic disorder of the first days of life caused by a marked deficiency of prothrombin[7] and stable factor.[8] There is strong evidence that PTC and Stuart Prower factors, which are associated with plasma thromboplastin formation, are also deficient in hemorrhagic disease of the newborn infant.[3,16]

Etiology—Prothrombin and Stable Factor Deficiency. Hemorrhagic disease of the newborn infant represents an exaggeration of prothrombin and stable factor deficiencies, events which normally occur in the first days of life. Prothrombin and stable factor values, already diminished at birth, fall sharply in the second to the fourth days, returning to normal spontaneously at seven to ten days.

Stable factor is more markedly reduced than prothrombin and accounts pri

These agents act as anticoagulants by interfering with the hepatic synthesis of prothrombin and similar factors During the first days of treatment with any of the dicoumarin group of drugs it is probable that stable factor deficiency is the main cause of the lengthened one stage prothrombin time [1]

Congenital Deficiencies Congenital deficiencies represent coagulation disorders resulting from isolated deficiencies of one of the factors of the prothrombin complex The bleeding manifestations are similar to those observed in patients with disorders in which multiple deficiencies exist and will be described later In the congenital deficiencies (prothrombin and labile stable and Stuart Prower factors) vitamin K therapy is uniformly unsuccessful

Idiopathic (Congenital) Hypoprothrombinemia Congenital prothrombin deficiencies are rare but authentic cases of failure to synthesize this protein have been reported There may a family history of idiopathic hypoprothrombinemia *with hemorrhages appearing in childhood and bleeding from mucous surfaces* and into skin and tissues Since the majority of patients with hypoprothrombinemia usually possess associated deficiencies it is important to rule out labile and stable factor involvement especially the latter [94] before the diagnosis of true hypoprothrombinemia is justified

Labile Factor Deficiency (Factor V Deficiency, Parahemophilia, Owren's Disease)

Originally described by Owren[56 8] and since by others [9] labile factor deficiency is detected by a prolonged one stage prothrombin time with undiluted plasma which is corrected when the patient's plasma is mixed with deprothrombinized (barium sulfate–absorbed) normal human plasma The coagulation time of whole blood is prolonged and the bleeding time may be prolonged or normal In patients with labile or stable factor deficiencies bleeding is usually from the mucous membranes and skin In both conditions the bleeding tendency dates from infancy but may not be manifested until later childhood after trauma or a surgical procedure such as tonsillectomy Many newborn infants have low levels of labile factor during the first two days of life which rise toward normal by the sixth day [63] A syndrome of congenital labile factor deficiency with syndactylism has been reported In this family with normal parents five of seven siblings had syndactylism and a bleeding tendency In three of the children a deficiency of the labile factor in the plasma was demonstrated [38]

Labile factor deficiency is a familial disease affecting both sexes and is transmitted as an incompletely dominant gene [5] It has also been suggested[63] that the plasma of the homozygous patient is almost entirely lacking in labile factor and the patient is a bleeder However the heterozygous person has only a partial deficiency and is usually not a bleeder The disease may be of variable intensity The only treatment of known value is transfusion with *fresh* normal blood or plasma because of the lability of the factor The abnormality is not affected by vitamin K Acquired labile factor deficiency occurs in persons with a variety of clinical conditions usually in association with prothrombin deficiency since both factors are affected simultaneously after surgical operations

and in the presence of liver disease leukemia malignancies pernicious anemia and abruptio placentae Factor V deficiency has been reported in patients with hemorrhagic scarlet fever (purpura fulminans)[69]

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marily for the prolonged one stage prothrombin time[43] The deficiency of these coagulation factors especially stable factor is more pronounced in the premature than in the full term infant and the return to normal takes place more slowly

Several factors contribute to the genesis of physiologic prothrombin and stable factor deficiencies the bacteria free nature of the newborn infant s intestinal tract which prevents vitamin K synthesis until intestinal flora are established with food ingestion liver immaturity a lack of a reserve supply of the vitamin and in some cases a depletion of vitamin K in the mother due to poor dietary intake poor absorption and impaired utilization due to liver dysfunction

Concentrations of 20 to 25 per cent of prothrombin and stable factor are critical values below which a potential hemorrhagic state exists Values of 5 per cent and lower occur in infants with hemorrhagic disease In the majority of patients bleeding occurs spontaneously during the period when the prothrombin and stable factor concentrations are normally reduced The incidence of hemorrhagic disease of the newborn infant is given variously as about 1 in 200 to 400 births but it appears much less commonly at present perhaps due to the widespread use of vitamin K

A deficiency of PTC (active in the first phase of coagulation) occurs in combination with prothrombin and stable factor deficiencies in the newborn period[82 115] PTC deficiency follows the pattern of the prothrombin complex in the first weeks of life with its most marked decrease about the third day of life and spontaneously increasing thereafter Aballi and co workers observed marked improvement in PTC concentration by administering vitamin K to full term infants but an inadequate response in the premature infant Because of the slowness with which prothrombin stable factor and PTC return to non hemorrhagic levels in the premature infant and because of the inadequacy of vitamin K therapy it may be safer to defer circumcision for a period of three to four weeks

Clinical and Laboratory Features Bleeding is rarely massive consisting of persistent oozing from the umbilical cord and mucous surfaces hematemesis hematuria gastrointestinal and vaginal bleeding petechiae ecchymoses and infrequently spontaneous central nervous system and meningeal hemorrhage Serious hemorrhage can occur from minor trauma Circumcision may result in slow and continued bleeding[30] There is suggestive evidence that the PTC deficiency is especially responsible for the prolonged coagulation time and the tendency to bleed encountered in patients with this disease

The platelet count is normal and anemia is proportional to blood loss In addition to prolonged prothrombin time prolonged coagulation and bleeding time occasionally occur but are not necessary for the diagnosis The presence of an associated vascular lesion is suggested by the prolonged bleeding time rarely positive tourniquet test and evidence of skin hemorrhages

Differential Diagnosis The stools of many infants are benzidine positive indicating the presence of blood A markedly prolonged prothrombin time differentiates hemorrhagic disease of the newborn infant from blood derived from maternal sources such as blood swallowed at birth or while nursing from fissured nipples In a grossly bloody stool this test is helpful but what is even more specific is the identification of the source of blood as to its maternal or fetal nature

The application of the alkali denaturation test distinguishes between maternal blood (adult type) and infant's blood (fetal type) the latter being more resistant to denaturation with alkali than adult hemoglobin [4] Anticoagulant therapy in the mother may provoke serious bleeding in the fetus and newborn infant Conditions other than the hypoprothrombinemias deserve consideration in the differential diagnosis Disorders of thromboplastin formation and afibrinogenemia can be eliminated by appropriate tests Oozing from the umbilical cord occurs in infants with hemorrhagic disease but is unusual in those with hemophilia Infants with congenital thrombocytopenic purpura congenital syphilis bacteremia and hemorrhagic manifestations of erythroblastosis fetalis also present clinical bleeding in the first days of life

Treatment The discovery of vitamin K by Dam[3] and of its relationship to prothrombin formation led to its use in pregnant women before delivery and in the infant at birth to prevent hypoprothrombinemia In recent years however doubt has been thrown on the value of vitamin K in the prevention [6] and management[4] of hemorrhagic disease of the newborn infant In the premature infant especially the shortage of vitamin K is not so important as the inadequate synthesis of the clotting factors (prothrombin and stable factor) caused by immaturity of the liver Occasionally prothrombin levels have been raised by vitamin K given to the mother or infant but this did not reduce the incidence of the disease There is ample evidence however that the administration of vitamin K to the newborn infant arrests the fall of prothrombin and stable factor although normal levels may not be achieved [13] In another series vitamin K administration corrected all the clotting defects (stable factor PTC and Stuart Prower factor deficiencies and hypoprothrombinemia) associated with hemorrhagic disease of the newborn infant [3] Until a more effective means of controlling hemorrhagic disease of the newborn infant with its potentially serious clinical manifestations becomes available vitamin K therapy still remains the agent of choice for prophylaxis and treatment

Vitamin K The large number of vitamin K preparations may be classified as those containing oil soluble vitamin K and the synthetic analogues many of which are water soluble One of the very effective natural vitamin K products K_1 phytonadione (Konakion * Mephyton†) can be given intramuscularly intravenously or as a tablet for oral use Two synthetic water soluble vitamin K preparations which are in common use are menadiol sodium bisulfate (Hykinone‡) and menadiol sodium diphosphate (Synkavite) They are available for oral use and subcutaneous intramuscular and intravenous injection

Excessive doses of vitamin K are to be avoided because of their known hemolytic action and tendency to cause hyperbilirubinemia especially in the premature infant It has therefore been recommended by the Council on Drugs of the American Medical Association[11] that as an equivalent of 1 mg of synthetic vitamin K_1 menadione is adequate to prevent hemorrhagic disease of the newborn infant

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*Roche Laboratories Nutley N J

†Merck Sharp & Dohme Inc Philadelphia Pa

‡Abbott Laboratories North Chicago Ill

The natural K_1 vitamins act more rapidly than the synthetic preparations and raise plasma prothrombin to therapeutic levels within two to four hours Phytonadione is of special value in threatening or actual hemorrhage Prophylaxis of hemorrhagic disease of the newborn infant is achieved by the use of phytonadione (Mephyton) or other natural or synthetic vitamin K (menadione) by giving the mother daily doses of 2 to 4 mg orally for one week before delivery or parenteral injection of an equivalent dose of one of the water soluble preparations several hours before delivery For the infant at birth phytonadione is effective when given intramuscularly in a 1 to 2 mg dose The water soluble forms of vitamin K (Hykinone Synkavite) are however completely adequate in the presence of vitamin K deficiency states

In our own institution normal newborn infants are not given vitamin K routinely A dose of 1 to 2 mg of vitamin K_1 (phytonadione) is given intramuscularly to all premature infants and infants in all weight groups exhibiting marked fetal distress and anoxia Routine administration of vitamin K to antepartum mothers has been discontinued We have not been impressed with any increased incidence of hemorrhagic disease of the newborn infants since discontinuance of vitamin K administration as a routine measure in prepartum mothers of full term infants

The danger of hyperbilirubinemia in the infant from excessive dosage of vitamin K given to the mother has been noted The parenteral administration of 72 mg of a vitamin K analogue (menadione sodium bisulfate Hykinone) during labor resulted in bilirubin levels in the newborn premature infant ranging from 21 to 47 mg per 100 ml with neurologic sequelae in two patients

Transfusions of blood or plasma are given to replenish prothrombin and stable Stuart Prower and PTC factors and in severely affected patients to combat anemia and shock Fresh human serum (in 5 ml amounts) is especially recommended as a therapeutic adjuvant in the severely affected patient because of its readily available content of stable factor which may be especially involved Although external bleeding can be controlled internal bleeding results in an appreciable mortality (estimated at 70 per cent)

Stuart Prower Factor Deficiency

The Stuart Prower factor[38 8 106] functions both in the formation of intrinsic and extrinsic thromboplastin Deficiencies of the factor have been found in patients with liver disease in newborn infants with hemorrhagic disease and in patients receiving coumarin compounds [3] The deficiency is transmitted as an incompletely recessive autosomal character In the heterozygote this factor is reduced on an average to 36 per cent and causes mild bleeding The homozygote has a moderately severe hemorrhagic disorder manifested by epistaxis hematomas and hemarthrosis A deficiency of Stuart Prower factor is an important part of the clotting defect of premature[106] and full term infants [3]

Multiple Defects

Capillary and Single Coagulation Factor Deficiency Vascular hemophilia (pseudohemophilia B) represents a dual hemostatic disorder in which a capillary defect responsible for clinical bleeding is combined with an AHG deficiency

The vascular defect alone is responsible for an increased bleeding time and often a positive tourniquet test Capillary disorders have also been found to coexist with either labile or stable factor deficiencies (factors V and VII respectively) with congenital afibrinogenemia and with double factor deficiencies[114]

Multiple Factor Deficiencies A combined deficiency of AHG and PTC[9] and of congenital labile factor with AHG deficiency has also been described[64]

DISORDERS DUE TO A DEFICIENCY OF FIBRINOGEN (PHASE 3 OF COAGULATION)

A deficiency of fibrinogen is a rare coagulation defect resulting in a profound disturbance of the blood clotting mechanism The complete deficiency is congenital whereas conditions with reduced amounts of fibrinogen (hypofibrinogenemia or fibrinogenopenia) are either congenital or acquired

Congenital Afibrinogenemia

Congenital afibrinogenemia is marked by a complete or practically complete and permanent absence of fibrinogen from the blood resulting in a hemorrhagic syndrome resembling hemophilia except that it affects either sex[9] The patient is frequently the offspring of a consanguineous marriage An incomplete recessive or semidominant inheritance[54] is postulated since low fibrinogen levels are occasionally found in the parents of affected children[40]

Bleeding is manifest from birth with excessive oozing from the umbilical cord or from the navel on separation of the cord and after circumcision Persistent bleeding follows trauma surgical procedures and the loss of deciduous teeth Excessive bruising and epistaxis are common In contrast to hemophilia hemarthroses are rare[3] Joint disability does not result even in this circumstance probably due to a lack of fibrin formation Except for the absence of fibrinogen the other phases of clotting are normal Despite incoagulable blood long intervals with freedom from hemorrhage are common[11] and the condition is usually associated with only a minor degree of disability

Congenital Hypofibrinogenemia

Reduced fibrinogen levels may be present in parents of children with afibrinogenemia in whom bleeding is absent or in patients with "constitutional fibrinogenemia" in whom there is a tendency toward abnormal bleeding

Acquired Fibrinogen Deficiency

Acquired fibrinogen deficiencies have been noted in patients with many conditions such as severe liver disease bone marrow involvement with invasion by leukemic or tumor cells polycythemia vera malignancies tuberculosis shock burns transfusion reactions[11] and obstetric complications

Hypofibrinogenemia in Patients With Congenital Heart Disease

A potential hemorrhagic diathesis in congenital cyanotic heart disease has been described Marked impairment of clot retraction is due to a combination of thrombocytopenia and low blood fibrinogen in the presence of a high hemato

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time Hence if the whole blood clotting time is prolonged and the Quick one stage prothrombin time shows no clotting or a very incomplete clot total or partial fibrinogen should be suspected [1] It has been estimated that if the prothrombin time of a given plasma sample is normal its fibrinogen level must be in excess of 100 mg per cent [11]

In hypofibrinogenemia the clotting time may be normal or slightly prolonged depending upon the content of fibrinogen With reduced fibrinogen levels clotting occurs promptly and retraction of the clot results in a relatively large volume of erythrocytes over which is suspended a very small mass Eventually the clot becomes detached and drops into the erythrocyte suspension by which it is obscured

Treatment of the Fibrinogen Deficiencies

The normal concentration of fibrinogen in the blood is 250 to 400 mg per 100 ml Only in the event of a hemorrhagic episode is treatment necessary and this consists of the administration of blood plasma and concentrated fibrinogen (Cohn fraction I) Approximately 0 2 to 0 3 gm of fibrinogen is present in 100 ml of plasma and about 0 7 to 0 9 gm in 1 pint of whole blood Concentrated human fibrinogen preparations are now in good supply and constitute the first choice of treatment In contrast to the labile antihemophilic globulin fibrinogen remains stable when stored In critical situations in which severe and persistent bleeding is associated with marked fibrinogen depletion substantial amounts of human fibrinogen may be given (up to 4 gm intravenously in children) It must be cautioned however that the use of fibrinogen carries a definite hazard of hepatitis

A critical blood level of 80 mg of fibrinogen per 100 ml is required for effective hemostasis and treatment should be directed toward achieving at least, this concentration Gitlin and Borges [9] determined immunochemically that one half of administered fibrinogen disappeared from the circulation in the first forty eight hours After the first two days the fibrinogen follows a logarithmic decay curve with a half life of four days indicating that the fundamental defect in these patients is a failure to synthesize adequate amounts of fibrinogen Fibrinogen labeled with radioactive iodine I^{131} shows a mean half life of 5 1 days (range 4 1 to 6 days) [3] A dose of 100 mg of fibrinogen per kilogram of body weight raises the fibrinogen concentration of a patient with afibrinogenemia to approximately 200 mg per 100 ml [11] With the use of plasma the fibrinogen component may remain in the circulation for as long as four days and with concentrated fibrinogen it may remain twice as long [4]

In patients with acute fibrinolysis treatment is urgent and consists of prompt administration of whole blood plasma and concentrated fibrinogen (fraction I) Use of ACTH and the steroid hormones has been suggested as a means of increasing antifibrinolysin activity[109] and controlling bleeding

CIRCULATING ANTICOAGULANTS

Naturally Occurring Anticoagulants The fluidity of the circulating blood depends upon the integrity of vascular endothelium and the presence of the

crit The slight to moderate prolongation of prothrombin time is not a cause of the hemorrhagic disorder Although these abnormal findings were present in patients in all age groups postoperative hemorrhage was chiefly a problem in adults and adolescents Children however with evidence of marked impairment revealed by hematologic tests underwent operation without hemorrhage In adults with polycythemia vera as well as in patients with secondary polycythemia of congenital heart disease the hemorrhagic tendency is attributed in large measure to fibrinogenopenia The treatment preoperatively in emergency situations in patients with polycythemia is liberal venesection together with the return of normal plasma to the patient [109]

Incoagulability of the blood as a result of hypofibrinogenemia is a well known complication of several obstetric conditions amniotic fluid infusion (embolism) premature separation of the placenta and prolonged retention of a dead fetus One of the explanations for the deficiency in fibrinogen is that a thromboplastin like material *associated with these complications* gains access to the maternal circulation resulting in fibrinogen consumption Another theory is that as fibrin is formed intravascularly it is destroyed by a fibrinolysin released as a result of shock [71]

Hypofibrinogenemia has been encountered among the sensitized mothers of severely ill and stillborn infants in the course of erythroblastosis It has been pointed out that the threat of developing afibrinogenemia in these patients may necessitate early delivery [19]

Fibrinolysis (Fibrinolytic Purpura)

The fibrinolytic process is one in which the living organism dissolves blood clots and disposes of fibrin [1-] This is effected through a complex enzymatic system *The activation of enzymes capable of destroying fibrin occurs acutely* in patients with trauma hemorrhagic shock extensive burns transfusion reactions and obstetric accidents and chronically in those with leukemia liver disease and disseminated carcinoma

Fibrinolysis accounts for recanalization of a blood vessel after hemostasis has been completed Disruption of the hemostatic mechanism in patients with carcinoma or after extensive surgery with severe bleeding manifestations (fibrinolytic purpura) results from enzymatic digestion of fibrin clot fibrinogen and other proteins involved in blood coagulation [108] In patients with hemorrhage following abruptio placentae and after surgery for cirrhosis of the liver the primary causative factor of afibrinogenemia is fibrinolysis [90]

Fibrinolysis depends upon the liberation into circulation of tissue kinases which activate preformed and inert precursors (profibrinolysins) In another terminology the precursor plasminogen is converted to the active proteolytic enzyme (plasmin) by various activation systems (fibrinokinase or tissue activators derived from tissues) [12] The mechanism of activation is obscure Excessive fibrinolysis frequently results in severe bleeding from multiple areas

Laboratory Findings in Fibrinogen Deficiencies

In complete afibrinogenemia the whole blood is incoagulable The absence of fibrinogen is confirmed by the failure of citrated blood or plasma to clot when thrombin is added and by the absence of a precipitate when the plasma is heated to 56 C [1] If the plasma remains clear afibrinogenemia exists since fibrinogen precipitates at about this temperature

The blood of patients with hemophilia shows a normal one stage prothrombin

may be necessary however in the face of continued bleeding although there is the risk of generating increased amounts of anticoagulant ACTH and steroids *have been of limited value However the anticoagulant has been known to* disappear spontaneously or the bleeding may be minimal despite its presence

SUMMARY OF REPLACEMENT THERAPY OF THE COAGULATION DISORDERS

The frequency of plasma administration depends upon the life span of the deficient factor in the patient s circulation loss of the factors through utilization the increased requirements with active bleeding consumption by internal hemorrhage or possibly by neutralization or destructive action of anticoagulants[98] When the critical level of a particular factor in a patient with any bleeding disorder (of the first or second stage of coagulation) approximates that observed in those with hemophilia due to AHG deficiency comparable amounts of plasma are given initially (10 ml per kilogram of body weight) The intervals between the *administration however may be increased* depending upon the survival of a particular factor Thus except for AHG the intervals of administration may be lengthened to twenty four to forty eight hours or longer for patients with PTC or PTA deficiency and for those with the hypoprothrombinemias

Fibrinogen may be administered in the form of plasma Since 100 ml of plasma supplies 10 mg of fibrinogen this dosage must be repeated to bring the total to 80 mg per cent or over as required for hemostasis The slow destruction in the blood stream reduces the need for frequent plasma administration

For surgery in patients with one of the hemophilias plasma is given thirty *minutes before the operation to be repeated every four to six hours in patients* with AHG deficiency and every twelve to twenty four hours in those with PTC or PTA deficiency After the first twenty four hours plasma is given to patients with AHG deficiency in accordance with the specifications of the schedule given previously in this chapter and every forty eight hours to seventy two hours to those with PTC or PTA deficiency The type of plasma to be used whether fresh or stored is indicated in the following list

1 Fresh whole blood or plasma
   - A Thrombocytopenic purpura (whole blood or platelet rich plasma collected in siliconized or plastic equipment
   - B Hemophilia (hemophilia A AHG deficiency)
   - C Parahemophilia (labile or factor V deficiency)
   - D von Willebrand s disease
     - (1) Vascular defect only (pseudohemophilia A)
     - (2) Vascular defect with AHG deficiency (vascular hemophilia pseudohemophilia B)

2 Fresh or stored whole blood or plasma
   - A Prothrombin deficiency
   - B PTC deficiency (hemophilia B Christmas factor)
   - C PTA deficiency
   - D Factor VII deficiency (stable factor)
   - E Stuart Prower factor deficiency

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natural anticoagulants plasma antithrombin heparin and a postulated antithromboplastin

Antithromboplastin has been described as a lipid inhibitor an excess of which has been held accountable for hemophilia [11, 18] It has been shown for instance that contact of hemophilic plasma with asbestos or glass will normalize its clotting by adsorption of the inhibitor On the other hand transfusion of hemophilic plasma treated by removal of the inhibitor in this manner or by extraction with ether still demonstrates a deficiency of AHG in in vivo experiments Nevertheless the presence of an inhibitor in hemophilic plasma cannot be discounted Proof of the presence of circulating anticoagulant as an intrinsic feature of the hemophilic defect within the circulation awaits further corroborative evidences [7]

Acquired Anticoagulants The majority of acquired inhibitors prevent the formation of thromboplastin either as a complication of hemophilia or causing a hemorrhagic diathesis resembling hemophilia [61] The best defined blood clotting inhibitors are those which appear in the blood of the patient with hemophilia following repeated transfusions This has been ascribed to an immunologic response to the antihemophilic globulin in blood or plasma Similar inhibitors of plasma thromboplastin component (PTC) have also been described [4]

Acquired hemophilia like disease based on circulating anticoagulants has also been recorded after pregnancy In one case the anticoagulant was detected in the patient s child during the first two and one half months of life indicating its transplacental transfer [48] Circulating anticoagulants have appeared in previously healthy male and female patients or in connection with an associated illness notably lupus erythematosus periarteritis nodosa rheumatoid arthritis tuberculosis and glomerulonephritis Hemorrhagic disease due to a rise in blood heparin or substances with heparin like activity has appeared after treatment with physical or chemical reagents such as nitrogen mustard and after exposure to ionizing radiation and probably explains the decreased coagulability of the blood in patients with anaphylaxis

The presence of a circulating anticoagulant contributes to the diagnosis of lupus erythematosus This abnormality may disappear fairly rapidly during steroid therapy [9a] In one patient with this disease severe bleeding was associated with hypoprothrombinemia plus an anticoagulant active against formed blood and tissue prothrombinase [94c]

Tests for Anticoagulants A screening test for the presence of clotting inhibitors is an integral part of a complete coagulation study Patients with circulating anticoagulant usually show prolonged clotting and recalcification time Although normal whole blood or plasma will correct a deficiency the presence of an anticoagulant in the patient s blood or plasma will prolong the normal clotting time The screening test consists of mixing various amounts of the patient s blood or plasma with normal blood or plasma and determining the clotting and recalcification times of the mixtures [104] Circulating anticoagulants can also be detected by the thromboplastin generation test by mixing either the patient s serum or adsorbed plasma with the corresponding normal reagent With an inhibitor little or no thromboplastin is generated [3]

Treatment Attempts to overcome the refractory state by repeated transfusions with massive amounts of blood have been of questionable success This measure

events in pediatric practice Prothrombin consumption and thromboplastin generation tests provide accurate information concerning basic defects in coagulation but are obviously not suited for routine use The most practical course is to obtain a detailed personal and family history of hemorrhage

As a general rule a hemorrhagic disorder is suspected when the degree of bleeding is out of proportion to the extent of trauma This circumstance usually becomes apparent by the time the child is walking without protection More complete studies are warranted in the patient with a history of severe and uncontrollable epistaxis easy bruising hematomas especially in protected areas of the body and excessive bleeding from cuts skin prick as for blood counts and injections and during the eruption or loss of deciduous teeth Importance is attached to a history of prolonged bleeding or transfusions after an operative procedure in a sibling or parent The absence of bleeding after a dental extraction of moderate severity or after tonsillectomy usually indicates the absence of congenital disease

The clotting time is only of value in the older child in whom a vein can be entered without difficulty Capillary tests are unreliable because of the amount of tissue juice containing thromboplastin which enters the peripheral blood sample Since a normal clotting time will be found in patients mildly affected with hemophilias and often in those with conditions with defects in prothrombin conversion the history becomes increasingly important Short of the use of more complicated tests the bleeding history is the most useful means of anticipating difficulty in patients with sporadic AHG and PTC deficiency in whom there is no family history (25 to 30 per cent of patients with hemophilia)

In addition to the history a complete physical examination and examination of the blood smear for platelets to rule out thrombopenic states at the time of routine preoperative hemoglobin determinations and white cell counts constitute effective screening devices For these reasons a careful history is essential and it has even been suggested that because of inherently misleading results[39] routine presurgery tests of bleeding and clotting times be abandoned

LABORATORY INVESTIGATION OF COAGULATION DISORDERS

General Considerations Investigation for a hemorrhagic disorder is usually prompted by the clinical history and physical findings which also give direction to the scope of the laboratory work up The tests consist of the routine coagulation procedures for screening and more elaborate devices for the detection of the specific causative defect

In evaluating the results of routine coagulation tests which may be the only ones available in a laboratory it should be remembered that patients with the mild bleeding disorders may show few or no abnormalities upon routine testing In such instances a careful history will compel attention toward the need for more intensive investigation

In the history of the patient or other members of his family those episodes are important in which bleeding from minor cuts and scratches and after dental extraction and tonsillectomy is prolonged with or without the need for trans

Data relative to plasma requirements in deficiencies of coagulation factors are as follows

1 Stable when stored
   A PTC
   B PTA
   C Prothrombin
   D Stable factor (factor VII)
   E Fibrinogen
2 Labile when stored
   A AHG
   B Labile factor (factor V)
3 Survival time of clotting factors in circulation of patient[*]
   A AHG—12 to 24 hours
   B PTC—48 to 72 hours
   C PTA—24 hours
   D Prothrombin—36 to 42 hours
   E Stable factor—24 to 36 hours
   F Labile factor—24 to 48 hours
   G Fibrinogen—4 days

EPISTAXIS

Recurrent nosebleeds constitute a common problem in pediatric practice They usually *occur spontaneously often at night and frequently in the course* of an upper respiratory infection They are usually controlled by simple measures such as digital pressure or cotton packs rimmed with petroleum jelly or soaked in Adrenalin but sometimes they require cauterization and nasal packing Bleeding however may be profuse and blood loss may be substantial

The localization and causation of nosebleeds assume importance when tonsillectomy and adenoidectomy are contemplated As an isolated symptom it is rare in patients with hemophilia In those with intractable nosebleeds pseudohemophilia should be considered The platelet count is usually normal in patients with idiopathic epistaxis and depressed in those in whom epistaxis accompanies leukemia aplastic anemia or conditions associated with hypersplenism such as Gaucher s disease Nosebleeds infrequently constitute the sole clinical feature of either idiopathic thrombocytopenic purpura or classic (AHG deficient) hemophilia Epistaxis may also occur in patients during the course of measles rheumatic fever systemic lupus erythematosus typhoid fever glomerulonephritis sickle cell anemia and Cooley s anemia

From present evidence it appears safe for patients with epistaxis without a history of abnormal bleeding to be operated upon without further tests This means that in neither the patient nor any member of his family is there a history of easy bruising or persistent bleeding after dental extraction circumcision immunization or eruption or loss of deciduous teeth Furthermore physical examination should reveal no petechiae purpura or ecchymoses

PROCEDURE FOR SCREENING POTENTIAL BLEEDERS

Adequate screening devices before surgery are especially important in infants and children since circumcision dental extraction and tonsillectomy are common

events in pediatric practice Prothrombin consumption and thromboplastin generation tests provide accurate information concerning basic defects in coagulation but are obviously not suited for routine use The most practical course is to obtain a detailed personal and family history of hemorrhage

As a general rule a hemorrhagic disorder is suspected when the degree of bleeding is out of proportion to the extent of trauma This circumstance usually becomes apparent by the time the child is walking without protection More complete studies are warranted in the patient with a history of severe and uncontrollable epistaxis easy bruising hematomas especially in protected areas of the body and excessive bleeding from cuts skin prick as for blood counts and injections and during the eruption or loss of deciduous teeth Importance is attached to a history of prolonged bleeding or transfusions after an operative procedure in a sibling or parent The absence of bleeding after a dental extraction of moderate severity or after tonsillectomy usually indicates the absence of congenital disease

The clotting time is only of value in the older child in whom a vein can be entered without difficulty Capillary tests are unreliable because of the amount of tissue juice containing thromboplastin which enters the peripheral blood sample Since a normal clotting time will be found in patients mildly affected with hemophilias and often in those with conditions with defects in prothrombin conversion the history becomes increasingly important Short of the use of more complicated tests the bleeding history is the most useful means of anticipating difficulty in patients with sporadic AHG and PTC deficiency in whom there is no family history (25 to 30 per cent of patients with hemophilia)

In addition to the history a complete physical examination and examination of the blood smear for platelets to rule out thrombopenic states at the time of routine preoperative hemoglobin determinations and white cell counts constitute effective screening devices For these reasons a careful history is essential and it has even been suggested that because of inherently misleading results[39] routine presurgery tests of bleeding and clotting times be abandoned

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In evaluating the results of routine coagulation tests which may be the only ones available in a laboratory it should be remembered that patients with the mild bleeding disorders may show few or no abnormalities upon routine testing In such instances a careful history will compel attention toward the need for more intensive investigation

In the history of the patient or other members of his family those episodes are important in which bleeding from minor cuts and scratches and after dental extraction and tonsillectomy is prolonged with or without the need for trans

fusions In the infant more than normal oozing of blood after circumcision and from cuts of the tongue and lips protracted oozing of blood from the gums during the *eruption of deciduous teeth and bleeding at the site of immunization* are noteworthy The presence of extensive bruising observed during physical examination is a sign of recurrent hemorrhage The bruising may represent a disorder in which platelets are deficient

Both hematologic and nonhematologic disturbances may give evidence of gross bleeding and must be differentiated from a basic bleeding tendency Epistaxis may be a feature of thrombocytopenic purpura and leukemia and hematemesis and melena may be features of Banti s disease Severe epistaxis a frequent occurrence in childhood deserves a complete coagulation study if it is associated with easy bruising and a suspicious bleeding history On the other hand epistaxis per se without other evidence of bleeding, or a negative family or personal history of bleeding has in our experience not justified more than the *routine laboratory examinations For all practical purposes a patient who* has undergone tonsillectomy or tooth extraction without evidence of abnormal bleeding may be considered not to have hemophilia or any other serious first phase coagulation defect

Screening Tests The screening tests for a suspected hemorrhagic disorder include coagulation time bleeding time clot retraction prothrombin time (one stage) complete blood count including platelet count and capillary fragility (tourniquet test) Since diagnosis choice of therapy and prognosis depend upon the results of laboratory methods meticulous attention to details is neces sary in these preliminary tests as in the more elaborate procedures (See Table 18)

Coagulation Time Blood is obtained by vein puncture Capillary coagulation tests are unreliable because blood obtained by skin puncture is mixed with tissue juice and therefore is unsuitable for determining the clotting time If the vein puncture is not accomplished immediately and if there is any undue manipu lation the procedure is repeated with another needle and vein The Lee and White technique is used One milliliter of blood is transferred into each of two clean dry Pyrex tubes measuring 13 by 100 mm and tests are carried out in a water bath kept at 37 C The first tube is tilted at half minute intervals until no blood flows when the tube is inverted The second tube is then similarly tilted until the blood is solidified The point at which the blood is solidified is designated as the coagulation time The normal range of coagulation time is five to twelve minutes

Bleeding Time Bleeding time is determined by the Duke method or by the Ivy method

DUKE METHOD With a No 11 Bard Parker *blade a small cut is made in the* ear lobe to a depth of 2 to 3 mm With a round piece of filter paper the blood is blotted off not wiped off every thirty seconds The blood should be permitted to flow onto the filter paper by capillarity The interval between the time the incision is made and the moment when bleeding stops represents the end point The range of normal is given as two to seven minutes falling between one and four minutes in the majority of patients

Table 18 Results of Routine Tests in Hemorrhagic States†*

Disorder	*Bleeding Time*	*Clotting Time*	*Clot Retraction*	*Tourniquet Test*	*Platelet Count*	*Prothrombin Time*
Platelet abnormalities						
Thrombocytopenia	A	N	A	A	A	N
Thrombasthenia	A	N	N or A	N or A	N	N
AHG deficiency	N	A	N	N	N	N
PTC deficiency	N	A	N	N	N	N
PTA deficiency	N	A‡	N	N	N	N
Prothrombin deficiency	N	N	N	N	N	A
Labile factor deficiency	N	N§	N	N	N	A
Stable factor deficiency	N	N	N	N	N	A
Fibrinogen deficiency	N	N	N	N or A	N	N or A
Vascular disorders	A	N	N	N or A	N	N
Stuart Prower factor deficiency	N	A	N	N	N	A

* From Schulman I and Smith C H Coagulation Disorders in Infancy and Childhood In Levine S Z (editor) Advances in Pediatrics vol 9 Chicago 1957 Year Book Publishers Inc p 231

† N normal A abnormal

‡ May be normal in mild to moderate degrees of deficiency

§ May be prolonged with marked deficiency

‖ Abnormal with marked deficiency since no end point present

Puncture of the ear lobe should be avoided in children with suspected hemophilia pseudohemophilia and severe thrombocytopenic purpura because bleeding may be so uncontrollable as to eventually require transfusions for hemostasis

IVY METHOD[6] A blood pressure cuff placed just above the bend of the elbow is kept at 40 mm pressure The surface of the forearm is cleansed with an antiseptic and then dried With a Bard Parker blade or a sterile spring lancet the skin is punctured to a depth of 2 to 3 mm and a width of 2 mm in the fleshy part of the forearm below the elbow The drops of blood are removed with a piece of filter paper every thirty seconds as is done in the Duke method The bleeding time is the time which elapses between the puncture of the forearm and the cessation of bleeding

This method has been recommended in children because persistent bleeding from the forearm can be more readily controlled by pressure than that from the ear lobe Despite the possibility of prolonged oozing the Duke test is still in current use and has been found reliable in most laboratories

Clot Retraction The clot in one of the tubes used in determining the clotting time is used for the clot retraction test The test should be facilitated by loosening the clot from the walls of the tube with a platinum wire or glass applicator The tube is stoppered placed in a water bath at 37 C and inspected at one two twelve and twenty four hours Normal clot retraction is usually complete in one or two hours

In the blood of patients with abnormal conditions contractility is minimal or absent after twenty four hours Clot retraction depends upon several factors the number of platelets the concentration of fibrinogen and the cell volume Adequate numbers of intact platelets are essential for clot retraction the higher the concentration of fibrinogen, the less the contraction Poor clot retraction is noticeable with counts below 80 000 per cubic millimeter No retraction is observed in patients with thrombocytopenia with counts below 20 000 per cubic millimeter and in those with thrombasthenia (normal number of platelets but impaired function) It is poor in patients with an excessive red cell mass Clot retraction is difficult to measure quantitatively although tests are available for that purpose

Platelet Count The reagent used is 3 8 per cent sodium citrate solution For the Rees Ecker modification brilliant cresyl blue is added An accurate platelet count is a prerequisite for the diagnosis of either thrombocytopenia or thrombocytosis Thrombocythemia refers to more persistently increased platelet levels than are observed in patients with thrombocytosis after splenectomy It is commonly seen in patients with myeloproliferative conditions such as chronic myelocytic leukemia Hodgkin s disease and Boeck s sarcoid Purpuric bleeding often with a prolonged bleeding time has been reported in patients with an excessively high platelet count This situation has been encountered most often in patients with polycythemia vera chronic myeloid leukemia and myeloid metaplasia[68] In those with thrombocytopenic purpura the blood smear presents a transparent appearance under the microscope due to reduced platelet numbers As in patients with many conditions which are accompanied by thrombocytopenia variations in morphology are likely to occur

Syndromes have been described in which abnormal bleeding has been attributed to qualitative platelet defects—the so called thrombocytopathic purpuras or thrombasthenia The platelets are usually normal rarely slightly reduced in number highly abnormal in appearance giant sized abnormally small bizarre in shape and agranular Glanzmann s thrombasthenia[1] is cited as an example of a syndrome in which hemorrhage occurs with a normal bleeding time platelet count and coagulation time but with poor clot retraction and abnormal platelet morphology With sharper separation of platelet functions it may be possible eventually to relate a bleeding disorder to a specific defect Patients have already been described in whom there is a defect in thromboplastin formation clot retraction and antiheparin activity of the blood—functions of platelets necessary for maintenance of normal hemostasis [116] Treatment of these patients is limited to transfusions of fresh or whole blood or platelet rich plasma

Tourniquet (Rumpel Leede) Test The tourniquet test serves as a measure of capillary resistance It consists of obstructing venous blood flow by a constricting band on the arm (blood pressure cuff) and recording the number of petechiae in a specified area distal to the obstruction The intracapillary pressure is increased sufficiently to demonstrate a state of latent increased permeability (capillary fragility) The test is commonly performed by maintaining the blood pressure midway between the systolic and diastolic pressures for three to five minutes After the cuff is removed the number of petechiae in a previously marked circular

area 5 cm in diameter on the flexor surface of the forearm a little below the bend of the elbow is counted A count above five is interpreted as positive Although the capillary resistance tends to parallel other manifestations of vascular inadequacy many exceptions are encountered so that a positive test is not always observed with a prolonged bleeding time and thrombocytopenia

Significance of Routine Coagulation Tests The screening tests thus far described provide unequivocal evidence of a single defect—namely an abnormality of platelets The combined abnormalities of clot retraction bleeding time the tourniquet test and of course a low platelet count support such a diagnosis

Markedly prolonged coagulation time is most commonly found in patients with deficiencies of the plasma thromboplastin precursors (AHG PTC and PTA) with severe fibrinogen deficiency and with circulating anticoagulants Mild degrees of AHG PTC and PTA deficiencies may coexist with entirely normal whole blood clotting time Abnormal results of the routine tests indicate the existence of a hemorrhagic state but do not give sufficient information for a specific diagnosis On the other hand normal results with these tests do not exclude a coagulation defect especially when the history is suggestive Further investigations carried out systematically are therefore necessary to detect minor degrees of depletion

Tests for Phase 1 of Coagulation

The tests for the first phase of coagulation are primarily designed to differentiate the three types of hemophilia classic hemophilia (hemophilia A) due to AHG deficiency Christmas disease (hemophilia B) due to PTC deficiency and PTA deficiency (hemophilia C) Platelet defects in number or quality can also be recognized by these tests

In patients with each type of hemophilia regardless of severity the platelet count bleeding time clot retraction prothrombin time and fibrinogen content are normal The severe grades of the hemophilias are characterized by markedly prolonged coagulation time In patients with milder types however the clotting time may be only slightly prolonged and in those with the mildest types it may be entirely normal With more elaborate and sensitive tests it is possible to detect lesser degrees of deficiency of the three plasma proteins (AHG PTC and PTA)

The tests currently employed in first phase coagulation abnormalities are the thromboplastin generation test and the prothrombin consumption test

Thromboplastin Generation Test Although the prothrombin consumption test detects moderate deficiencies of thromboplastin it is normal in patients with mild deficiencies of the factors (AHG PTC or PTA) that enter into its formation The thromboplastin generation test described by Biggs and Douglas[18] has proved to be a more sensitive test of thromboplastin formation defects It not only permits the detection of slight degrees of abnormality but more important it differentiates one type of abnormality from another a distinction not provided by the whole blood clotting time or prothrombin consumption test Specific deficiencies of antihemophilic globulin plasma thromboplastin component and plasma thromboplastin antecedent can thus be ascertained When

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*Table 19 Constituents of "Reagents" Prepared From Normal Blood**

Reagent	Phase 1	Phase 2	Phase 3
Whole plasma†	AHG PTC PTA ST P factor	Prothrombin Labile factor Stable factor ST P factor	Fibrinogen
Adsorbed plasma	AHG PTA	Labile factor†	Fibrinogen
Serum	PTC PTA ST P factor	Stable factor ST P factor	———

*Modified from Schulman I and Smith C H Coagulation Disorders in Infancy and Childhood In Levine S Z (editor) Advances in Pediatrics vol 9 Chicago 1957 Year Book Publishers p 231

†While labile factor appears in whole and adsorbed plasma and influences the thromboplastin generation tests it is usually assayed in Phase 2 Stuart Prower factor deficiency also gives Phase 1 and 2 abnormalities and may be assayed in both phases However in Phase 2 it is separated from stable factor deficiency by the Stypven test mentioned in the text

Table 20 Effects of Normal Plasma Normal Deprothrombinized Plasma and Normal Serum on the Coagulation Abnormalities in AHG PTC and PTA Deficiencies

Reagent Added to Patient's Plasma	Deficiency		
	AHG	PTC	PTA
Normal plasma	Corrects	Corrects	Corrects
Normal adsorbed plasma	Corrects	No correction	Corrects
Normal serum	No correction	Corrects	Corrects

From Schulman I and Smith C H Coagulation Disorders in Infancy and Childhood In Levine S Z (editor) Advances in Pediatrics vol 9 Chicago 1957 Year Book Publishers Inc p 231

tests in which plasma is used 0 025M calcium chloride is generally employed The factors present in each of these preparations are given in Table 19

Platelets in Thromboplastin Generation Test—Thrombasthenia and Thrombopathia [31, 66a, 116] It has been taken for granted that the platelets are normal ac

carried out in patients with disease of varying clinical severity the amount of thromboplastin formed bears some relation to clinical severity

With this test the progressive activity of *plasma thromboplastin is evaluated* by incubating at 37 C a mixture of adsorbed barium sulfate plasma (normally containing antihemophilic globulin AHG and plasma thromboplastin antecedent PTA) serum (normally containing plasma thromboplastin component PTC plasma thromboplastin antecedent PTA and Stuart Prower factor) platelets (or substitute) and calcium chloride At regular intervals measured samples of the incubation mixture are added to normal plasma (substrate) The thromboplastin generated in this incubation mixture causes clotting of the plasma and the speed of clotting is a measure of thromboplastin formation When all the first stage coagulation factors are present in normal amounts, the thromboplastin produced by the generating mixture after three to seven minutes incubation is sufficiently potent to achieve a plasma clotting time of eight to ten seconds

The thromboplastin generation test is similar to the prothrombin time inasmuch as both utilize the property of thromboplastin to shorten the clotting time of recalcified plasma

Preparation of Coagulation Factors for Thromboplastin Generation Test Except for fibrinogen which can be measured quantitatively the clotting factors involved in the production of thromboplastin and of the accessory factors in prothrombin conversion can be identified by analyzing their distinctive behavior and interaction in especially devised clotting systems With the use of minimal suspensions which selectively adsorb some factors in plasma and not others the knowledge that certain factors are present in plasma and absent in serum and the knowledge of the effect of storage upon plasma and serum factors it is possible to determine the presence or absence of factors in each stage of coagulation In testing for deficiencies in the first stage advantage is taken of the fact that adsorption removes several factors from normal plasma but leaves AHG and factor V (labile factor) intact

PLASMA In many of the coagulation studies plasma rather than whole blood is used It is prepared with one of the anticoagulants most frequently sodium oxalate When obtained from normal blood plasma contains all the coagulation factors in each phase of coagulation

ADSORBED PLASMA By treating the oxalated plasma with tricalcium phosphate gel or barium sulfate deprothrombinized plasma results These adsorbing agents remove not only prothrombin but also PTC stable factor and Stuart Prower factor (ST P) leaving AHG PTA labile factor and fibrinogen For citrated blood aluminum hydroxide is used as the adsorbing reagent

SERUM If normal whole blood is allowed to clot in a test tube antihemophilic globulin (AHG) labile factor prothrombin and fibrinogen are consumed Normal serum then contains PTC PTA stable factor and Stuart Prower factor (ST P) These serum factors are stable when stored

PLATELETS In many of the tests performed with these blood products suspensions of normal platelets are required More recently platelet substitutes such as human brain or soy bean cephalin have been used

CALCIUM CHLORIDE Calcium chloride is required to institute clotting in all

and values of more than 15 to 20 per cent to result in a normal thromboplastin generation Hence abnormal tests will result only when the antihemophilic content of plasma falls below these levels

Prothrombin Consumption Test The prothrombin consumption test is performed on serum after clotting has occurred in a manner similar to the one stage test for prothrombin time of plasma with one addition Since serum contains stable factor but not fibrinogen or labile factor these must be supplemented in order to determine the residual prothrombin after clotting The additional factors required are present in plasma which has been adsorbed with barium sulfate or tricalcium phosphate The treated plasma contains labile factor and fibrinogen but it is free of prothrombin

With a 1 ml serologic pipette 0 1 ml of serum to be analyzed (residual after clotting) is blown into a mixture of 0 1 ml of calcium chloride 0 1 ml of adsorbed plasma (source of labile factor and fibrinogen) and 0 1 ml of thromboplastin reagent (as in the test for plasma prothrombin time) The time required for the clot to form is accurately measured

The normal prothrombin consumption time is over twenty five seconds[44] thus indicating very little residual prothrombin The usual range of residual prothrombin in serum is given as 0 to 25 per cent Usually 75 per cent is converted to thrombin In patients with disorders of phase 1 the serum prothrombin time is below twenty five seconds and in severely affected patients as short as twelve to fourteen seconds In principle the prothrombin consumption test is similar to the Quick prothrombin time test inasmuch as the former measures the prothrombin remaining in serum and the latter that which is present in the plasma before coagulation

Interpretation of Prothrombin Consumption Test The prothrombin consumption test is a quantitative determination of the residual prothrombin in serum after clotting has occurred When normal blood in a test tube clots sufficient thromboplastin is formed in the initial phase of coagulation to convert virtually all of the prothrombin to thrombin so that at completion of the process there is little or no prothrombin left in the serum If all the prothrombin is utilized and little or none is left in the serum it can be taken for granted that adequate amounts of thromboplastin have been formed and that this first phase of coagulation is relatively normal On the other hand abnormalities of this phase are indicated by the presence of a large amount of residual prothrombin in the serum after clotting indicating the formation of inadequate amounts of thromboplastin required to convert prothrombin to thrombin Prothrombin activity of the serum is determined one hour after coagulation since thrombin continues to be formed after the blood or plasma has clotted After this interval practically all the prothrombin has been consumed The prothrombin consumption test though useful in detecting defects affecting the formation of thromboplastin is less sensitive than the thromboplastin generation test

Tests for Phase 2 of Coagulation

Prothrombin Complex Deficiencies In carrying out in vitro tests prothrombin factor V factor VII and the Stuart Prower factor must be present in sufficient

cording to the screening tests this having been previously established by normal platelet count clot retraction bleeding time and tourniquet test However in patients with platelet dysfunction the qualitative deficiency will be reflected in an abnormal thromboplastin generation test when the patient's platelets are placed in a system with normal adsorbed plasma and normal serum The test is corrected with normal platelets or platelet substitutes

The terms thrombasthenia and thrombopathia are often applied interchangeably to conditions in which platelets are qualitatively defective although normal in numbers In both conditions there is a severe bleeding tendency Thrombasthenia refers to a defective function of platelets in clot retraction thrombopathia (thrombocytopathia) refers to a defect in platelets in thromboplastin formation Thrombasthenia has been further characterized as an inability of platelets to form pseudopods and a lack of spreading in plasma and serums resulting in a disturbance in clot retraction [3b] In thrombocytopathia pseudopod formation and spread are normal but the platelets are giant in size and thromboplastin generation and prothrombin consumption are disturbed Thrombopathia has often been used as an all inclusive term for any qualitative platelet defect

The thromboplastin generation test can be modified to provide quantitative estimates of AHG activity and of other hemophilic factors [91]

A rapid screening test for thromboplastin generation has recently been reported by Hicks and Pitney [48] It is a useful and rapid method for eliminating those specimens in which further investigation is unnecessary The principle of the test is the recalcification of diluted whole plasma in the presence of a platelet substitute Thromboplastin generation is determined by adding samples of the incubation mixture to high spun normal recalcified plasma

Mutual Correction Tests The various hemophilias may be differentiated simply by testing for mutual correction in mixtures of blood or plasma from the patient with the unknown defect and from patients whose defects have been identified Blood or plasma from a patient with AHG deficiency will correct abnormal coagulation in a test tube of blood from patients with plasma thromboplastin component and plasma thromboplastin antecedent defects but not from another with AHG deficiency The same principle applies for correction of PTA deficient blood and plasma by AHG or PTC but not by PTA samples and for the correction of PTC deficient blood by AHG and PTA but not by PTC sample The difficulties in mutual correction tests are that a panel of blood samples from patients with known deficiencies is not always available hence the advantage of the precise diagnostic facilities of the thromboplastin generation test

Comparative Value of Laboratory Tests in Detecting Thromboplastin Deficiency Antihemophilic globulin is normally present in plasma in a range of 50 to 170 per cent Intermediate degrees of bleeding occur between the almost negligible levels of AHG in patients with classic hemophilia and the lower limit of normal The variable sensitivity of the tests enumerated for phase 1 abnormalities must therefore be considered in the diagnosis of hemophilia Thus as little as 1 per cent antihemophilic globulin is sufficient to produce a normal whole blood clotting time 3 to 5 per cent to yield a normal prothrombin consumption

prothrombin time by the addition of serum the patient lacks stable factor; if there is no improvement by adding both reagents (deprothrombinized plasma and serum) the patient has a deficiency of prothrombin itself.

Normal plasma, serum, and adsorbed plasma (with barium sulfate, hence deprothrombinized) contain the following factors of the prothrombin complex: normal plasma—prothrombin and labile, stable, and Stuart Prower factors; normal serum—stable factor and Stuart Prower factor; and adsorbed plasma—labile factor.

The differentiation of the prothrombin complex deficiencies is indicated in Table 21. Not included in the table is Stuart Prower factor deficiency, which follows the same pattern as stable factor (factor VII) deficiency.

Table 21. Differentiation of Prothrombin Complex Deficiencies

Deficient Factor Causing Abnormal (Prolonged) Prothrombin Time	*Effect of Addition of*		
	Normal Plasma	*Serum*	*Adsorbed Plasma*
Prothrombin	Corrects	No correction	No correction
Labile factor	Corrects	No correction	Corrects
Stable factor	Corrects	Corrects	No correction

Other differential tests for labile factor and stable factor deficiencies are based on the fact that when oxalated plasma is stored, labile factor disappears rapidly, whereas stable factor and prothrombin remain in high concentration. A reagent for determining the stable factor deficiency can also be prepared by the passage of plasma through a 30 per cent asbestos filter, which removes stable factor but retains prothrombin and labile factor.

Combined Prothrombin and Stable Factor Deficiency. If the patient's plasma does not correct the prolonged prothrombin time with aged plasma (contains stable factor but no labile factor), labile factor is deficient. If labile factor is present and the addition of small amounts of serum (contains abundant stable factor) only partially corrects the prothrombin time, then presumably both prothrombin and stable factor are deficient.

Stuart Prower Factor. Investigation of patients with a long Quick prothrombin time led to the discovery of a defect heretofore ascribed to a deficiency of factor VII.[34, 41] The Stuart Prower factor is present in normal plasma and is relatively heat and storage stable. This factor is now known to be essential both for normal plasma thromboplastin production and conversion of prothrombin to thrombin by its interaction with tissue extract. The Stuart Prower factor and factor VII are both adsorbed by tricalcium phosphate and other similar adsorbing agents. A deficiency of the Stuart Prower factor results in an abnormal thromboplastin generation test. It can be differentiated from factor VII when Stypven

amounts to convert prothrombin to thrombin and give a *normal* prothrombin time These factors collectively are frequently designated as the prothrombin complex A failure to effect conversion to thrombin *may result from* a deficiency of one or a combination of these *factors It should* again be emphasized that these are in vitro tests designed *to isolate* defects of any one or a combination of factors in the laboratory

Prothrombin Time (Plasma Prothrombin Time) The one stage test of Quick[93] has been found *most useful in* determining disturbances of the second phase of coagulation (the *conversion of* prothrombin to thrombin after activation by thromboplastin) *By* adding an excess of tissue extract (thromboplastin) and optimal *amounts* of calcium to measured samples of citrated or oxalated plasma prothrombin activity *is measured* by the time required for coagulation to occur *The one stage* prothrombin time serves as a specific indicator of phase 2 involve*ment and* includes the entire prothrombin complex—prothrombin and the ac*celerator agents* labile stable and Stuart Prower factors In our laboratory the *normal* prothrombin time by this method is eleven to fifteen seconds It should *be remembered* that no definitive prothrombin time can be stated which is the *same for* every laboratory Since the value obtained depends upon the potency of *the* thromboplastin used a normal control must be simultaneously determined *In terms of* prothrombin activity by comparison on a curve from suitable plasma *dilutions* the normal range is from 70 to 120 per cent

An elevated prothrombin time may reflect any single or combined deficiency *of* prothrombin factor VII factor V Stuart Prower factor and fibrinogen (absence of end point) An unusually low plasma content of fibrinogen may also account for a prolonged one stage reading but this value can be determined chemically A normal prothrombin time serves therefore incidentally as evidence of a normal fibrinogen content of plasma With the help of *simple correction* tests a deficiency of either labile or stable factor can be readily *determined*

The one stage prothrombin test is performed as follows The thromboplastin used is *generally obtained from* rabbit brain or rabbit lung Many excellent well standardized thromboplastin preparations are commercially available This test requires exacting technique the patient s plasma must be obtained in a standard manner the concentration of calcium chloride *must be exact* the temperature must be controlled at 37 C a stop watch is used for timing *and controls* are carried out With most techniques 0 1 ml of thromboplastin is then added *Timing is* started at this point and the clotting time is recorded Commercial preparations *are available in* which the thromboplastin and calcium are already mixed in proper amounts (Simplastin*) *The test then* requires only one step Preparations of control plasma for testing thromboplastic reagent are also commercially available (Diagnostic Plasma Protrol†) *As* previously stated with most techniques the one stage prothrombin times are usually twelve to fourteen seconds

Differential tests can be carried out in conjunction with the one stage prothrombin time to determine a deficiency of factor V factor VII prothrombin or Stuart Prower factor If there is correction of the Quick prothrombin time by the addition of the patient s plasma to deprothrombinized plasma (barium sulfate—absorbed) the patient lacks labile factor if there is correction of the Quick

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*Warner Chilcott Laboratories Morris Plains N J

†Knickerbocker Biologics Inc New York N Y

11 Alexander B Goldstein R Rich L Le Bolloc'h A G Diamond L K and Borges W Congenital Afibrinogenemia a Study of Some Basic Aspects of Coagulation Blood 9 843 1954
12 Astrup T Fibrinolysis in the Organism Blood 11 781 1956
13 Barnett C P Hemorrhagic Diathesis Due to Factor VII Deficiency A M A Arch Int Med 99 280 1957
14 Bell W N and Alton H G A Brain Extract as a Substitute for Platelet Suspensions in the Thromboplastin Generation Test Nature 174 880 1954
15 Bidwell E The Purification of Bovine Antihaemophilic Globulin Brit J Haemat 1 35 1955
16 Bidwell E The Purification of Antihaemophilic Globulin From Animal Plasma Brit J Haemat 1 386 1955
17 Biggs R Inhibitory Effect of Hemophilic Plasma Brit J Haemat 4 192 1958
18 Biggs R G and Douglas A S The Thromboplastin Generation Test J Clin Path 6 23 1953
19 Biggs R Douglas A S Macfarlane R G Dacie J V Pitney W R Merskey C and O Brien J R Christmas Disease a Condition Previously Mistaken for Haemophilia Brit M J 2 1378 1952
20 Biggs R Eveling J and Richards G Assay of Antihaemophilic Globulin Activity Brit J Haemat 1 20 1955
21 Biggs R and Macfarlane R G Human Blood Coagulation and Its Disorders ed 2 Oxford 1957 Blackwell Scientific Publications
22 Biggs R and Macfarlane R G Haemophilia and Related Conditions a Survey of 187 Cases Brit J Haemat 4 1 1958
23 Biggs R and Macfarlane R G Diagnosis of the Haemophiloid States Acta haemat 20 118 1958
23a Bolton F C and Clarke J E A Method of Assaying Christmas Factor Its Application to the Study of Christmas Disease (Factor IX Deficiency) Brit J Haemat 5 296 1959
23b Braunsteiner H and Pakesch F Thrombocytoasthenia and Thrombocytopathia—Old Names and New Diseases Blood 11 965 1956
24 Brinkhous K M Physiopathology of Hemophilia Acta haemat 20 125 1958
25 Brinkhous K M Langdell R D Penick G D Graham J B and Wagner R H Newer Approaches to the Study of Hemophilia and Hemophiloid States J A M A 154 481 1954
26 Brinkhous K M Penick G D Langdell R D Wagner R H and Graham J B Physiologic Basis of Transfusion Therapy in Hemophilia A M A Arch Path 61 6 1956
27 Brinkhous K M Smith H P and Warner E D Plasma Prothrombin Level in Normal Infancy and in Hemorrhagic Disease of the Newborn Am J M Sc 193 475 1939
28 Caffey J Pediatric X ray Diagnosis ed 3 Chicago 1956 Year Book Publishers Inc
29 Chen T I and Tsai C The Mechanism of Haemostasis in Peripheral Vessels J Physiol 107 280 1948
30 Clifford S H Hemorrhagic Disease of the Newborn J Pediat 14 333 1939
31 Crosby W H and Stefanini M Pathogenesis of the Plasma Transfusion Reactions With Special Reference to the Blood Coagulation System J Lab & Clin Med 40 374 1952
32 Dam H Dyggve H Larsen H and Plum P The Relation of Vitamin K Deficiency to Hemorrhagic Disease of the Newborn In Levine S Z (editor) Advances in Pediatrics vol 5 Chicago 1952 Year Book Publishers Inc p 129
33 Dacie D M M Defect of Antihemophilic Globulin in von Willebrand s Disease A M A Am J Dis Child 90 561 1955 (abst)
34 Davidson C S Epstein R D Miller G F and Taylor F H L Hemophilia a Clinical Study of Forty Patients Blood 4 489 1949
35 deGruchy G C Clinical Haematology in Medical Practice Springfield Ill 1958 Charles C Thomas Publisher p 542

(Russell's viper venom) combined with cephalin replaces tissue thromboplastin in the prothrombin test An abnormal prothrombin time is then obtained with Stuart Prower factor deficiency in contrast to a normal prothrombin time with plasma deficient in factor VII Factor VII is not needed in the thromboplastin generation test whereas the Stuart Prower factor is a necessary ingredient The clotting time in Stuart Prower factor deficiency is slightly prolonged Stuart Prower levels were found to be low in premature newborn infants at birth with an increase toward normal by the sixth day of life whether or not vitamin K_1 was given to the infants at birth [100a]

Tests for Phase 3 of Coagulation

Fibrinogen Deficiencies With adequate thrombin formation fibrinogen is converted to solid fibrin Fibrinogen occurs in plasma in a concentration of approximately 250 to 400 mg per 100 ml A threshold concentration of at least 60 mg per 100 ml is necessary for clotting to be detectable [1 1] Many methods are available for clinical quantitation of fibrinogen after its separation from plasma One of the simplest methods for clinical use is to measure the turbidity of a suspension of salt precipitated fibrinogen with a spectrophotometer [46] A simple test for determining the presence of fibrinogen in coagulable blood is the addition of thromboplastin or thrombin In patients with fibrinogenopenia the blood will remain incoagulable

REFERENCES

1 Aballi A J Banus V L deLamerens S and Rozengvaig S Coagulation Studies in the Newborn Period Alterations of Thromboplastin Generation and Effects of Vitamin K in Full Term Infants A M A J Dis Child 94 589 1957
2 Aballi A J Banus V L deLamerens S and Rozengvaig S Coagulation Studies in the Newborn Period Hemorrhagic Disease of the Newborn A M A J Dis Child 97 524 1959
3 Aballi A J Banus V L deLamerens S and Rozengvaig S Coagulation Studies in the Newborn Period Deficiency of Stuart Prower Factor as a Part of the Clotting Defect of the Newborn A M A J Dis Child 94 549 1959
4 Apt L and Downey W S Jr Melena Neonatorum The Swallowed Blood Syndrome a Simple Test for the Differentiation of Adult and Fetal Hemoglobin in Bloody Stools J Pediat 47 6 1955
5 Ackroyd J F Role of Platelets in Coagulation Thrombosis and Haemostasis With Some Observations on Platelet Dysfunction Including Thrombasthenia Brit M Bull 11 21 1955
6 Aggeler P M White S C Glendening M B Page E W Leake T B and Bates C Plasma Thromboplastin Component (PTC) Deficiency a New Disease Resembling Hemophilia Proc Soc Exper Biol & Med 79 692 1952
7 Alexander B Clotting Factor VII (Proconvertin) Synonymy Properties Clinical and Clinolaboratory Aspects New England J Med 260 1218 1959
8 Alexander B and Goldstein R Dual Hemostatic Defect in Pseudohemophilia J Clin Invest 29 795 1950
9 Alexander B and Goldstein R Parahemophilia in Three Siblings Am J Med 13 255 1952
10 Alexander B Goldstein R Landwehr G and Cook C D Congenital SPCA Deficiency a Hitherto Unrecognized Coagulation Defect With Hemorrhage Rectified by Serum and Serum Fractions J Clin Invest 30 596 1951

59 Hill J M and Speer R J Combined Hemophilia and PTC Deficiency Blood 10 357 1955
60 Hjort P Rapaport S I and Owren P A Evidence That Platelet Accelerator (Platelet Factor I) is Adsorbed Plasma Proaccelerin Blood 10 1139 1955
61 Hougie C Circulating Anticoagulants Brit M Bull 11 16 1955
61a Hougie C Reactions of Stuart Factor and Factor VII With Brain and Factor V Proc Soc Exper Biol & Med 101 132 1959
62 Hougie E Barrow E M and Graham J B Stuart Clotting Defect Segregation of an Hereditary Hemorrhagic State From the Heterogenous Group Heretofore Called Stable Factor (SPCA Proconvertin Factor VII) Deficiency J Clin Invest 36 485 1957
63 Israels L G Zipursky A and Sinclair C Factor V Levels in the Neonatal Period Pediatrics 15 180 1955
64 Iversen T and Bastrup Madsen P Congenital Familial Deficiency of Factor V (Parahaemophilia) Combined With Deficiency of Antihaemophilic Globulin Brit J Haemat 2 265 1956
65 Ivy A C Shapiro P R and Melnick P Bleeding Tendency in Jaundice Surg Gynec & Obst 60 781 1935
66 *Jackson D P Hartmann R C and Conley C L Clot Retraction as a Measure of* Platelet Function Clinical Disorders Associated With Qualitative Platelet Defects (*Thrombocytopathic Purpura*) Bull Johns Hopkins Hosp 93 370 1953
66a Johnson S A *Monto R W and Caldwell M J A New Approach to the Thrombocy*topathies Thrombocytopathy A Thrombosis et diathesis haemorrhagica 2 279 1958
67 Jordan H H Hemophilic Arthropathies Springfield Ill 1958 Charles C Thomas Publisher
68 Kingsley C S Familial Factor V Deficiency the Pattern of Heredity Quart J Med 23 323 1954
68a Koller F Symposium What Is Hemophilia? Is Hemophilia a Nosologic Entity? Blood 9 286 1954
69 Koller F Gasser C Krusi G and de Muralt G Purpura fulminans nach Scharlach mit Factor V Mangel und Antithrombinuberschuss Acta haemat 4 33 1950
70 Koller F Loeliger A and Duckert F Experiments on a New Clotting Factor (Factor VII) Acta haemat 6 1 1951
70a Kurkcuoglu M and McElfresh A E The Hageman Factor Determinations of Its Concentration During the Neonatal Period and Presentation of a Case of Hageman Factor Deficiency J Pediat 57 61 1960
71 Larkin I M and Philipp E E Further Experience With Hypofibrinogenemia of Pregnancy J Obst & Gynaec Brit Emp 64 215 1957
72 Larrieu M J and Soulier J P Deficit en facteur antihemophilique A chez une fille associe a un trouble du saignement Rev hemat 8 361 1953
73 Lewis J H and Ferguson J H Afibrinogenemia Report of a Case A M A Am J Dis Child 88 711 1954
74 Lewis J H Ferguson J H and Arends T Hemorrhagic Disease With Circulating Inhibitors of Blood Clotting Anti-AHF and Anti-PTC in Eight Cases Blood 11 846 1956
75 Lewis J H Ferguson J H Spaugh E Fresh J W and Zucker M B Acquired Hypoprothrombinemia Blood 12 84 1957
76 Loeliger A *and Koller F Behavior of Factor VII and Prothrombin in Late Pregnancy* and in Newborn Acta haemat 7 157 1952
77 Lucey J F and Dolan R G Injection of a Vitamin K Compound in Mothers and Hyperbilirubinemia in the Newborn Pediatrics 22 605 1958
78 MacAusland W R Jr and Gartland J J The Treatment of Acute Hemophilic Hemarthrosis Report on the Use of Hyaluronidase New England J Med 247 755 1952
79 Macfarlane R G The Bleeder Brit M J 1 1080 1955

36 Denson K W Studies of the Prower Factor a Blood Coagulation Factor Which Differs From Factor VII Brit J Haemat 4 313 1958
37 De Palma A F Management of Hemophilic Arthropathy—Hemophilia and Hemophilioid Diseases International Symposium Chapel Hill 1957 University of North Carolina Press
38 de Vries A Matoth P and Shamir Z S Familial Congenital Labile Factor Deficiency With Syndactylism Investigation on the Mode of Action of the Labile Factor Acta haemat 5 129 1951
39 Diamond L K and Porter F S The Inadequacies of Routine Bleeding and Clotting Times New England J Med 259 1025 1958
39a Didisheim P Ferguson J H and Lewis J H Hemostatic Data in Relatives of Hemophiliacs A and B A M A Arch Int Med 101 347 1958
40 Didisheim P and Lewis J H Congenital Disorders of the Mechanism for Coagulation of Blood Pediatrics 22 478 1958
40a Dische F E and Benfield V Congenital Factor VII Deficiency Haematological and Genetic Aspects Acta haemat 21 257 1959
41 Doses of Water Soluble Vitamin K Analogues in Hemorrhagic Disease of the Newborn J A M A 164 1331 1957
42 Douglas A S Antihemophilic Globulin Assay Following Plasma Infusions in Hemophilia J Lab & Clin Med 51 850 1958
43 Douglas A S and Davies P Hypoprothrombinemia in the Newborn Arch Dis Childhood 30 509 1955
44 Dreskin O H Prothrombin Consumption Test a Simplified Technique Am J Clin Path 22 140 1952
45 Duckert F Fluckiger P Matter M and Koller F Clotting Factor X Physiologic and Physio Chemical Properties Proc Soc Exper Biol & Med 90 17 1955
46 Fowell A H Turbidometric Method of Fibrinogen Assay Results With the Coleman Junior Spectrophotometer Am J Clin Path 25 340 1955
47 Fresh J W Ferguson J H Stamey C Morgan F M and Lewis J H Blood Prothrombin Proconvertin and Proaccelerin in Normal Infancy Questionable Relationships to Vitamin K Pediatrics 19 241 1957
48 Frick P G Hemophilia Like Disease Following Pregnancy With Transplacental Transfer of an Acquired Circulating Anticoagulant Blood 8 598 1953
49 Frick P and McQuarrie I Congenital Afibrinogenemia Pediatrics 13 44 1954
50 Gitlin D and Borges W H Studies on the Metabolism of Fibrinogen in Two Patients With Congenital Afibrinogenemia Blood 8 679 1953
51 Glanzmann E Hereditäre hamorrhagische thrombasthenie Ann paediat 68 113 1918
52 Gordon R R and Dean T Foetal Deaths From Antenatal Anticoagulant Therapy Brit M J 2 719 1955
53 Graham J B Barrow E M and Hougie C Stuart Clotting Defect the Genetic Aspects of a New Hemorrhagic State J Clin Invest 36 497 1957
53a Hammond J D S and Verel D Observations on the Distribution and Biological Half Life of Human Fibrinogen Brit J Haemat 5 431 1959
53b Hardisty R M The Reaction of Blood Coagulation Factor With Brain Extract Brit J Haemat 1 323 1955
54 Hardisty R M and Pinniger J L Congenital Afibrinogenemia Further Observations on the Blood Coagulation Mechanisms Brit J Haemat 2 139 1956
55 Hartmann R C Hemorrhagic Disorder Occurring in Patients With Cyanotic Congenital Heart Disease Bull Johns Hopkins Hosp 91 49 1952
56 Hay J D Hudson F P and Rodgers T S Vitamin K in the Prevention of Haemorrhagic Disease of the Newborn Lancet 1 423 1951
57 Henry F I Rosenthal R L and Hoffman I Spontaneous Hemorrhages Caused by Plasma Thromboplastin Antecedent Deficiency J A M A 162 727 1956
58 Hicks N D and Pitney W R A Rapid Screening Test for Disorders of Thromboplastin Generation Brit J Haemat 3 227 1957

95 Ratnoff O D and Colopy J E A Familial Hemorrhagic Trait Associated With Deficiency of a Clot Promoting Fraction of Plasma J Clin Invest 34 602 1955
96 Ratnoff O D and Rosenblum J M Role of Hageman Factor in the Initiation of Clotting by Glass Evidence That Glass Frees Hageman Factor From Inhibition Am J Med 25 160 1958
97 Rosenthal M C A Mechanism of Plasma Refractoriness in Hemophilia—Hemophilia and Hemophilioid Diseases International Symposium Chapel Hill 1957 University of North Carolina Press
98 Rosenthal M C The Therapy of Disorders of Coagulation J Chron Dis 6 383 1957
99 Rosenthal R L Properties of Plasma Thromboplastin Antecedent in Relation to Blood Coagulation J Lab & Clin Med 45 123 1955
100 Rosenthal R L Dreskin O H and Rosenthal N A New Hemophilia Like Disease Caused by Deficiency of a Third Plasma Thromboplastin Factor Proc Soc Exper Biol & Med 82 171 1953
101 Schulman I and Currimbhoy Z Hemorrhagic Disorders Pediat Clin North America May 1957
102 Schulman I Currimbhoy Z Smith C H Erlandson M E Schorr J B Fort E and Wehman J Phosphatides as Platelet Substitutes in Blood Coagulation Ann New York Acad Sc 75:195 1958
103 Schulman I and Smith C H Hemorrhagic Disease in an Infant Due to Deficiency of a Previously Undescribed Clotting Factor Blood 8 794 1952
104 Schulman I and Smith C H Coagulation Disorders in Infancy and Childhood In Levine S Z (editor) Advances in Pediatrics vol 9 Chicago 1957 Year Book Publishers Inc
105 Schulman I Smith C H Erlandson M Fort E and Lee R E Vascular Hemophilia a Familial Hemorrhagic Disease in Males and Females Characterized by Combined Antihemophilic Globulin Deficiency and Vascular Abnormality Pediatrics 18 347 1956
106 Schulz J and van Creveld S Stuart Prower Factor in Newborn Infants Etudes neonatales 7 133 1958
106a Schulz J and van Creveld S Stuart Prower Factor in Newborn Infants In Brinkhous K M (editor) Hemophilia and Other Hemorrhagic States International Symposium Rome Chapel Hill N C 1959 University of North Carolina Press p 167
107 Singer K and Ramot B Pseudohemophilia Type B Hereditary Hemorrhagic Diathesis Characterized by Prolonged Bleeding Time and Decrease in Antihemophilic Factor A M A Arch Int Med 97 115 1956
108 Stefanini M Fibrinolysis and Fibrinolytic Purpura Blood 7 1044 1952
109 Stefanini M and Dameshek W The Hemorrhagic Disorders New York 1955 Grune & Stratton Inc
110 Taylor F H L Davidson C S Tagnon H J Adams M A MacDonald A H and Minot G R Studies in Blood Coagulation the Coagulation Properties of Certain Globulin Fractions of Normal Human Plasma in Vitro J Clin Invest 24 698 1945
111 Telfer T P Denson K W and Wright D R A New Coagulation Defect Brit J Haemat 2 308 1956
112 Tocantins L M Hemophilic Syndromes and Hemophilia Blood 9 281 1954
113 Tocantins L M and Holburn R R The Coagulation of Blood Methods of Study New York 1955 Grune & Stratton Inc p 178
114 Valberg L S and Brown G M Haemorrhagic Capillary Disorder Associated With Antihaemophilic Globulin Deficiency Medicine 37 181 1958
115 van Creveld S Baker H Messing T Sjplema J J and Smits C A A M Thromboplastin Formation in the Blood of the Newborn Infant Études neonatales 3 217 1954
116 van Creveld S Ho L K and Veder H A Thrombopathia Acta haemat 19 199 1958
116a van Creveld S and Mochtar I A von Willebrand's Disease a Plasma Deficiency Cause of the Prolonged Bleeding Time Ann paediat 194 37 1960

79a Medal L S and Lisker R Circulating Anticoagulants in Disseminated Lupus Erythematosus Brit J Haemat 5 284 1959
80 Milstone J H The Chain Reaction of the Blood Clotting Mechanism in Relation to the Theory of Hemostasis and Thrombosis Blood 4 1290 1949
81 Matter M Newcomb R F Melly A and Finch C A Vascular Hemophilia the Association of a Vascular Deficiency With a Deficiency of Antihemophilic Globulin Am J M Sc 232 421 1956
82 McElfresh A L Sharpsteen J R and Akabane T The Generation of Thromboplastin and Levels of Plasma Thromboplastin Component of the Blood of Infants Pediatrics 17 870 1956
83 Merskey C The Occurrence of Hemophilia in the Human Female Quart J Med 20 299 1951
83a Miller S P Congenital Deficiency of Proconvertin a Clinical and Laboratory Report Blood 14 1322 1959
84 Neel J V and Schull W J Human Heredity Chicago Ill 1954 University of Chicago Press
84a Nilsson I M Blomback M Jorpes E Blomback B and Johansson S A von Willebrand's Disease and Its Correction With Human Plasma Fraction I O Acta med scandinav 159:179 1957
84b Nilsson I M Blomback M Thulen A and v Francken I Carriers of Hemophilia A a Laboratory Study Acta med scandinav 165:357 1959
85 Owen C A and Cooper T E Parahemophilia A M A Arch Int Med 95 194 1955
86 Owren P A Coagulation of the Blood Investigations on a New Clotting Factor Acta med scandinav (suppl 194) p 1 1947
87 Owren P A Prothrombin and Accessory Factors Am J Med 14 201 1953
88 Penick G D and Brinkhous K M Relative Stability of Plasma Antihemophilic Factor (AHF) Under Different Conditions of Storage Am J M Sc 232 434 1956
89 Perry S and Craddock C G Jr Platelet Adsorptive Properties and Platelet Extracts in Thromboplastin Generation Blood 8 177 1958
90 Phillips L L and Skrodelis V The Fibrinolytic Enzyme System in Normal Hemorrhagic and Disease States J Clin Invest 37 965 1958
91 Pitney W R The Assay of Antihemophilic Globulin (AHG) in Plasma Brit J Haemat 2 250 1956
91a Pitney W R and Arnold B J Plasma Antihemophilic Factor (AHG) Concentrations in Families of Patients With Haemorrhagic States Brit J Haemat 5 184 1959
91b Pitney W R and Arnold B J Laboratory Findings of Patients Suffering From von Willebrand's Disease Brit J Haemat 6 81 1960
92 Pool J G and Robinson J Observations on Plasma Banking and Transfusion Procedures for Haemophilic Patients Using a Quantitative Assay for Antihaemophilic Globulin (AHG) Brit J Haemat 5 24 1959
92a Prichard R W and Vann R L Congenital Afibrinogenemia Report on a Child Without Fibrinogen and Review of the Literature A M A Am J Dis Child 88 703 1954
93 Quick A J Hemorrhagic Diseases Philadelphia 1957 Lea & Febiger
94 Quick A J Pisciotta A V and Hussey C V Congenital Hypoprothrombinemic States A M A Arch Int Med 95 2 1955
94a Raccuglia G and Neel J V Congenital Vascular Defect Associated With Platelet Abnormality and Antihemophilic Factor Deficiency Blood 15 807 1960
94b Rabiner S F Winick M and Smith C H Congenital Deficiency of Factor VII Associated With Hemorrhagic Disease of the Newborn Report of a Case Pediatrics 25 101 1960
94c Rapaport S I Ames S B and Duvall B J A Plasma Coagulation Defect in Systemic Lupus Erythematosus Arising From Hypoprothrombinemia Combined With Antiprothrombinase Activity Blood 15 212 1960

95 Ratnoff O D and Colopy J E A Familial Hemorrhagic Trait Associated With Deficiency of a Clot Promoting Fraction of Plasma J Clin Invest 34 602 1955
96 Ratnoff O D and Rosenblum J M Role of Hageman Factor in the Initiation of Clotting by Glass Evidence That Glass Frees Hageman Factor From Inhibition Am J Med 25 160 1958
97 Rosenthal M C A Mechanism of Plasma Refractoriness in Hemophilia–Hemophilia and Hemophilioid Diseases International Symposium Chapel Hill 1957 University of North Carolina Press
98 Rosenthal M C The Therapy of Disorders of Coagulation J Chron Dis 6 383 1957
99 Rosenthal R L Properties of Plasma Thromboplastin Antecedent in Relation to Blood Coagulation J Lab & Clin Med 45 123 1955
100 Rosenthal R L Dreskin O H and Rosenthal N A New Hemophilia Like Disease Caused by Deficiency of a Third Plasma Thromboplastin Factor Proc Soc Exper Biol & Med 82 171 1953
101 Schulman I and Currimbhoy Z Hemorrhagic Disorders Pediat Clin North America May 1957
102 Schulman I Currimbhoy Z Smith C H Erlandson M E Schorr J B Fort E and Wehman J Phosphatides as Platelet Substitutes in Blood Coagulation Ann New York Acad Sc 75 195 1958
103 Schulman I and Smith C H Hemorrhagic Disease in an Infant Due to Deficiency of a Previously Undescribed Clotting Factor Blood 8 794 1952
104 Schulman I and Smith C H Coagulation Disorders in Infancy and Childhood In Levine S Z (editor) Advances in Pediatrics vol 9 Chicago 1957 Year Book Publishers Inc
105 Schulman I Smith C H Erlandson M Fort E and Lee R E Vascular Hemophilia a Familial Hemorrhagic Disease in Males and Females Characterized by Combined Antihemophilic Globulin Deficiency and Vascular Abnormality Pediatrics 18 347 1956
106 Schulz J and van Creveld S Stuart Prower Factor in Newborn Infants Études néonatales 7 133 1958
106a Schulz J and van Creveld S Stuart Prower Factor in Newborn Infants In Brinkhous K M (editor) Hemophilia and Other Hemorrhagic States International Symposium Rome Chapel Hill N C 1959 University of North Carolina Press p 167
107 Singer K and Ramot B Pseudohemophilia Type B Hereditary Hemorrhagic Diathesis Characterized by Prolonged Bleeding Time and Decrease in Antihemophilic Factor A M A Arch Int Med 97 715 1956
108 Stefanini M Fibrinolysis and Fibrinolytic Purpura Blood 7 1044 1952
109 Stefanini M and Dameshek W The Hemorrhagic Disorders New York 1955 Grune & Stratton Inc
110 Taylor F H L Davidson C S Tagnon H J Adams M A MacDonald A H and Minot G R Studies in Blood Coagulation the Coagulation Properties of Certain Globulin Fractions of Normal Human Plasma in Vitro J Clin Invest 24 698 1945
111 Telfer T P Denson K W and Wright D R A New" Coagulation Defect Brit J Haemat 2 308 1956
112 Tocantins L M Hemophilic Syndromes and Hemophilia Blood 9 281 1954
113 Tocantins L M and Holburn R R The Coagulation of Blood Methods of Study New York 1955 Grune & Stratton Inc p 176
114 Valberg L S and Brown G M Haemorrhagic Capillary Disorder Associated With Antihaemophilic Globulin Deficiency Medicine 37 181 1958
115 van Creveld S Baker H Nussing T Sipkema J J and Smits C A A M Thromboplastin Formation in the Blood of the Newborn Infant Études néonatales 3 217 1954
116 van Creveld S Ho L K and Veder H A Thrombopathia Acta haemat 19 199 1958
116a van Creveld S and Mochtar I A von Willebrand's Disease a Plasma Deficiency Cause of the Prolonged Bleeding Time Ann paediat 194 37 1960

117 van Creveld S Nagel C G M Nienhuis J H Miranda S I and Kie T S Thromboplastin Formation in the Blood of the Newborn Études neonatales 3 135 1954
118 van Creveld S and Paulssen M M P Isolation and Properties of a Third Clotting Factor in Blood Platelets Lancet 1:23 1952
119 Vaughan, V C III Management of Hemolytic Disease of the Newborn Infant J Pediat 54 566 1959
120 von Willebrand E A Über hereditäre Pseudohämophilie Acta med scandinav 76 521 1931
120a Welsh J F Serum Transfusion in the Treatment of Deficiency of Plasma Thromboplastin Component Am J Clin Path 33 118 1960
121 Wolman I J Laboratory Applications in Clinical Pediatrics New York, 1957 Blakiston Co
121a Wright I S Concerning the Functions and Nomenclature of Blood Clotting Factors With a Preliminary Report of the Profile of Blood Clotting Factors in Young Males Ann Int Med 51 841 1959
122 Zucker M B Platelet Agglutination and Vasoconstriction as Factors in Spontaneous Hemostasis in Normal Thrombocytopenic Heparinized and Hypoprothrombinemic Rats Am J Physiol 148:275 1947
123 Zucker M B and Rapport M M Identification and Quantitative Determination of Serotonin in Platelets the Source of Serum Serotonin Fed Proc 3 170 1954

CHAPTER 27

The Purpuras

The purpuras embrace a miscellaneous group of blood disorders which have in common skin hemorrhages with or without bleeding into the mucous membranes and other sites and into internal organs Extravasations of blood may vary from small pinpoint petechiae to large ecchymotic areas Purpura is a variable symptom complex resulting from a diversity of etiologic agents often with thrombocytopenia and with other conditions in which there are no platelet changes but which have striking clinical features Purpuras can therefore be classified as thrombocytopenic in which case they are classified as either idiopathic or secondary to well defined disorders or as nonthrombopenic such as Henoch Schonlein disease

THROMBOCYTOPENIC PURPURAS

Following are the thrombocytopenic purpuras due to either quantitative or qualitative platelet abnormalities

1 With low platelet count (thrombocytopenic states)
  A Idiopathic thrombocytopenic purpura
  B Symptomatic thrombocytopenic purpura
    (1) Caused by infections
      (a) Sepsis
      (b) Subacute bacterial endocarditis
      (c) Typhus
      (d) Measles
      (e) German measles
      (f) Scarlet fever
      (g) Hemorrhagic smallpox
    (2) Caused by chemicals drugs physical agents (x rays radioactive substance)
    (3) Associated with blood disorders
      (a) Secondary to marrow infiltration leukemia neoplasm fibrosis
      (b) Anemias (hypoplastic and aplastic) in association with autoimmune hemolytic anemia pernicious anemia megaloblastic anemia of infancy
      (c) Secondary to splenic hyperfunction in various diseases—Gaucher s disease Banti s syndrome Felty s syndrome lymphomas lupus erythematosus hypersplenism
      (d) Miscellaneous blood disorders—congenital and neonatal thrombocytopenia thrombocytopenia associated with hemangioma thrombotic (thrombohemolytic) thrombocytopenic purpura infectious mononucleosis massive blood transfusions

2. With normal platelet count
   - A. Allergic purpura
   - B. Congenital vascular defects
   - C. Purpura simplex
   - D. Idiopathic pulmonary hemosiderosis
   - E. Infections such as meningococcemia (Waterhouse-Friderichsen syndrome) and scarlet fever (purpura fulminans)
   - F. Drugs and chemical agents and avitaminosis (scurvy)
   - G. Thrombasthenia and thrombopathia

Thrombocytopenic states may also be classified on the basis of the bone marrow content of megakaryocytes.[88]

1. With normal or increased megakaryocytes in the marrow
   - A. Idiopathic thrombocytopenic purpura
   - B. Congenital (neonatal) thrombocytopenic purpura
   - C. Secondary to splenic hyperfunction in various diseases (Gaucher's disease, Banti's syndrome, Felty's syndrome, lymphomas, lupus, hypersplenism)
   - D. Drug sensitization (such as caused by quinidine)
2. With decreased or absent megakaryocytes in the marrow
   - A. Secondary to marrow replacement by tumor, leukemia, fibrosis
   - B. Aplastic anemia
   - C. Marrow damage due to drugs, chemical agents (such as chloramphenicol), radiation, etc.
   - D. Congenital (neonatal) thrombocytopenic purpura

In patients with amegakaryocytic thrombocytopenia the megakaryocytes are strikingly reduced in number. Most often the disappearance of megakaryocytes is due to replacement and filtration of bone marrow by abnormal cells, as occurs in patients with leukemia and lymphosarcoma and other types of neoplasms. It will be noted that congenital thrombocytopenia may be associated with either decreased or normal numbers of megakaryocytes.

Idiopathic Thrombocytopenic Purpura (ITP, Werlhof's Disease, Purpura Hemorrhagica)

Idiopathic thrombocytopenic purpura is a disease of unknown etiology characterized by a hemorrhagic tendency resulting from a marked reduction in the number of platelets with extravasation of blood into the skin, mucous membranes and subcutaneous tissues.

Pathogenesis. Many theories have been suggested for the pathogenesis of idiopathic thrombocytopenic purpura to explain its central features, the thrombocytopenia, increased capillary permeability and the role of the spleen.[90] These include the movement of platelets from the circulation to the site of injured vessels with corresponding reduction in the peripheral blood,[97] decreased platelet formation due to an abnormal humoral factor elaborated by the spleen and inhibiting megakaryocytic function,[18] and the destruction of platelets after their selective sequestration by the spleen.[6]

More recently interest has shifted to an immunoallergic mechanism as an etiologic factor. Information from two sources suggested the possibility that an immunologic reaction might be involved in idiopathic thrombocytopenic purpura. The observation that infants born to mothers with this disease were often pur-

puric was ascribed to the transmission of an immune body across the placenta In addition Evans and co workers[8, 9] presented evidence for the existence of a relationship between acquired hemolytic anemia and primary thrombocytopenic purpura They noted that acquired hemolytic anemia with sensitization of the red cells is often accompanied by thrombocytopenia and that primary thrombocytopenia in turn was frequently accompanied by red cell sensitization with or without hemolytic anemia Since acquired hemolytic anemia had been shown to be due to an autoantibody Evans and associates suggested that primary thrombocytopenic purpura was due to a thrombocyte autoantibody

Harrington and co workers[47] *have demonstrated that an immunologic mechanism* is responsible for the low platelet count in many patients with idiopathic thrombocytopenic purpura Platelet agglutinins have been demonstrated in vitro in the plasma of many patients whose thrombocytopenia was of the idiopathic variety A factor presumably identical to this platelet agglutinin is capable of inducing thrombocytopenic purpura and altering megakaryocytes in normal recipients of this plasma In terms of this concept the spleen is involved in pathogenesis by removing sensitized platelets and producing platelet agglutinin in variable amounts Some of the antibody is made in the spleen the major portion is made elsewhere

In spite of the evidence that idiopathic thrombocytopenic purpura is based on an immunologic mechanism circulating platelet antibodies have not been universally found with standard procedures

That a capillary defect undoubtedly plays a role in the etiology of the disease is reflected in the prolonged bleeding time and purpuric manifestations On the other hand there is no true correlation between the bleeding time or hemorrhage and the degree of thrombocytopenia A factor in pathogenesis is the demonstration[60] that normal capillary contraction does not occur in patients with thrombocytopenic purpura and other hemorrhagic states

The concept of a "thrombocytopoietin" for platelet formation comparable to erythropoietin for red cells is suggested by a case report of a child with chronic idiopathic thrombocytopenic purpura in whom a marked increase in the number of platelets followed transfusion with fresh or stored blood or plasma[74] The pathogenesis of the purpura in this patient appeared to be related to a congenital deficiency of a platelet stimulating factor present in normal plasma The factor appears to act by stimulating megakaryocyte maturation and platelet production[74] The existence of a human thrombopoietin system regulating thrombopoiesis was also described in thrombocythemic human serum[1b]

Clinical Manifestations Idiopathic thrombocytopenic purpura occurs in one of two major types—an acute self limited form or a chronic protracted disease with occasional remissions The disease occurs most commonly in children and young adults with the greater frequency in younger age groups and usually between 2 and 8 years of age[66] In the adult the disease occurs four to five times as frequently in females as in males in children no sex difference is noted

The acute form which is common in children has a sudden onset frequently after an upper respiratory infection or following measles[34] rubella[1, 30a, 87] chicken pox[113] mumps or infectious mononucleosis It is usually difficult to relate the

disease to an infection or to a drug employed during treatment An increased incidence of allergic manifestations has been found among relatives of patients and in about 15 per cent of the patients[1]

The chief complaint is easy bruising Cutaneous purpura is either spontaneous or secondary to minor trauma The size of the hemorrhage varies from small petechiae of pin head size to large ecchymoses The initial period is often marked by widely scattered petechiae with purpura with large extravasations of blood following later The color changes from red to purplish to brown with progressive liberation of pigments Nosebleeds gingival bleeding and bleeding into and from the oral mucous membranes gastrointestinal tract kidneys and vagina frequently accompany the purpura especially at the onset Petechiae may be found in the subconjunctivae and in the palate The interior surfaces of the lower extremities the buttocks and especially over bony prominences such as the ribs scapulae shoulders legs and pubic area are commonly affected Hematomas form over the lower extremities Hematuria, hematemesis melena and hemarthroses are infrequent Menorrhagia at or shortly after puberty may be the first indication of idiopathic thrombocytopenic purpura with a deficiency in blood platelets and impaired capillary resistance appearing soon after[40] Intracranial hemorrhage is uncommon in children and constitutes the most serious complication of this disease occurring usually early in its course With the exception of purpuric manifestations there are few physical findings The spleen is not palpable or is barely so If significant splenomegaly is present other diagnoses should be considered notably leukemia lymphosarcoma Banti's syndrome or other conditions associated with hypersplenism

Laboratory Data Examination of the bone marrow and peripheral blood are usually sufficient to establish the diagnosis

Blood Findings The most significant finding in the laboratory examination of the patient with thrombocytopenia is a platelet count usually below 60 000 per cubic millimeter and frequently below 20 000 This may be confirmed by the sparsity of platelets in the blood smear in which the relatively few platelets seen are often single large and abnormal in shape The bleeding time is characteristically prolonged the tourniquet test is positive and clot retraction is poor to absent The prothrombin time and whole blood clotting time are normal The existence of a true disturbance in the coagulation mechanism in idiopathic thrombocytopenic purpura is demonstrated by an abnormal prothrombin consumption test which is indicative of impaired thromboplastin formation A normochromic anemia with reticulocytosis occurs if there is epistaxis or if bleeding into the urinary tract or gastrointestinal canal is severe A moderate leukocytosis with an increase in granulocyte forms occurs if purpura is excessive

Bone Marrow Findings In patients with idiopathic thrombocytopenic purpura the principal abnormality is confined to the megakaryocytes In patients with active bleeding normoblastic and often myeloid hyperplasia may be present The megakaryocytes are normal or increased in number and show reduced platelet formation The megakaryocytes vary in size amount of cytoplasm and lobulation of the nucleus Agranular vacuolated degenerated and immature forms are present.



Fig 46 *A* Smear from normal bone marrow Note mature megakaryocyte containing large lobulated nucleus Cytoplasm is granular with platelets in the process of formation Masses of platelets are chiefly grouped about the periphery of the cell *B* Intermediate megakaryocytes These are usually found in increased numbers in the bone marrow of patients with idiopathic thrombocytopenic purpura Cytoplasm is more abundant and less granular than that shown in *A* and platelet differentiation is either absent (upper cell) or slight (lower cell) (Courtesy Dr Robert L Rosenthal New York N Y)

In one study[18] platelet production was found in only 8 to 19 per cent of all megakaryocytes and following splenectomy in 69 to 85 per cent of all cells. As the disease enters the chronic phase, megakaryocytes, although more mature, still show a reduction in granulation and platelet formation. The massive liberation of platelets after splenectomy has been interpreted as an indication that the spleen inhibits platelet formation from the megakaryocyte and prevents their delivery in the circulating blood.[90]

Despite these considerations, bone marrow examination cannot be regarded as diagnostic of idiopathic thrombocytopenic purpura. Its chief function lies in the exclusion of disease with which idiopathic thrombocytopenic purpura can be confused, principally aplastic anemia and leukemia and less often lymphosarcoma and other infiltrative diseases.

Several studies have shown that there is no consistent pattern of megakaryocytes that can be correlated with the course or prognosis of idiopathic thrombocytopenic purpura.[6] In patients with a complete or almost complete absence of megakaryocytes, however, splenectomy is contraindicated and an underlying condition should be sought. Bone marrow examinations should be repeated to establish the existence of decreased megakaryocytes. Eosinophilia in the bone marrow, frequently observed in this disease and regarded as a favorable prognostic sign for spontaneous recovery,[0] has not been confirmed.

Diagnosis. It is important initially to distinguish between nonthrombocytopenic and thrombocytopenic purpura. To establish a diagnosis of idiopathic thrombocytopenic purpura, secondary causative factors such as chemical and physical agents, blood disorders and infections which depress platelets must be eliminated. Bone marrow aspiration is an aid in differentiating idiopathic thrombocytopenia from aplastic anemia and leukemia, since it may resemble these conditions closely in some phase of the respective diseases. In patients with thrombocytopenia, megakaryocytes are normal or increased and red cell and granulocytic precursors are normal. In those with aplastic anemia and leukemia all elements including the megakaryocytes are depressed (amegakaryocytic) and in the latter the bone marrow is infiltrated with blast forms. The thrombocytopenia of metastatic carcinoma and lymphosarcoma is also associated with an amegakaryocytic bone marrow.

In patients with idiopathic thrombocytopenia the spleen is not enlarged. Where moderate or marked splenomegaly exists in association with thrombocytopenia, other conditions such as Gaucher's disease, Banti's syndrome and reticuloendotheliosis are to be considered. Sudden epistaxis or gastrointestinal hemorrhage together with thrombocytopenia in an infant whose spleen has become enlarged may indicate reticuloendotheliosis.

Disseminated lupus erythematosus may exist as an underlying disturbance manifesting itself solely as thrombocytopenic purpura without rash or joint manifestations. Examination for L.E. cells has therefore been recommended as a routine test in all patients with thrombocytopenic purpura, preferably before steroids are administered. The development of overt disseminated lupus erythematosus following splenectomy for idiopathic thrombocytopenic purpura has been interpreted as an indication that the intact spleen exerts an inhibitory influence

in preventing the full expression of the latent disease [19, 0] Although the development of lupus erythematosus has been estimated to have occurred in as many as 25 per cent of all patients splenectomized for thrombocytopenic purpura [0] this sequence has not as yet been observed in our clinic nor in another childrens clinic [84]

Course and Prognosis A spontaneous remission occurs in 75 per cent of infants and children as compared with 25 to 30 per cent of adults The disease may run its course in a few weeks without recurrence The majority of children recover completely within three months usually in the first six weeks and 10 to 15 per cent recover within four to six months Spontaneous and permanent recovery may not occur for six months to one year during which time purpura remits and relapses with varying degrees of thrombocytopenia with each attack About 10 per cent of children develop the chronic form and of these approximately 85 per cent recover with splenectomy In chronically affected patients particularly a constant search must be made for an underlying disease particularly aplastic anemia in which the definitive features of the complete disease may not be manifest for a year or more

Treatment Fresh blood platelet rich plasma platelet transfusions corticosteroids and splenectomy are used in the treatment of thrombocytopenic purpura Early treatment consists of transfusions of fresh whole blood in the event of severe anemia from blood loss and the administration of adrenocortical steroids and less commonly ACTH Although intracranial hemorrhage is rare in children rest or modified activity is advisable when platelet counts drop below 30 000 per cubic millimeter Subsequent attendance at school and resumption of a normal regime depend upon cessation of cutaneous hemorrhages and an increase in the number of platelets

Transfusions In the nonanemic patient whose bleeding is not controlled by steroids and tends to be progressive viable platelets can be obtained either as platelet rich plasma by sedimentation or slow centrifugation of fresh whole blood or as platelet concentrates obtained from several units of blood Despite the natural tendency of the acute disease in most children to run a favorable course with a spontaneous remission the possibility of cerebral hemorrhage or other occult internal bleeding justifies the administration of steroids from the onset of the illness [100] It has also been shown that nearly all deaths occur in the first month of the disease [4] Furthermore it is possible that steroids may shorten the disease by accelerating the onset of a remission

Steroids As to the choice of a drug adrenocortical steroids and ACTH are both effective in the relief of bleeding symptoms both by an early hemostatic effect mediated by action on the blood vessel wall[30] and by a later rise in platelets Of the various agents prednisone or like substance is most useful because of ease of administration and the fact that it lends itself more readily to regulation of dosage An increase in the number of platelets in the patient with acute idiopathic thrombocytopenic purpura with a favorable prognosis occurs within a week or ten days after the initiation of therapy The mechanism of the platelet increase is not apparent one of the explanations offered is that prednisone re

duces the concentration of antibody responsible for platelet destruction or megakaryocytic inhibition [0]

The dosage of steroids is variable and is adjusted for the age of the patient severity of the disease and the individual tendency to develop the side effects The amount of prednisone is similar to that given for acute leukemia (see chapter 23)—namely 15 to 20 mg daily for patients under 2 years of age and 20 to 60 mg for older children depending upon their age For triamcinolone and methyl prednisolone compounds four fifths of this dose is given (see Chapter 23) Like all adrenocortical steroids the drug is given in four divided doses at six hour intervals In the infant the dose must be carefully adjusted to avoid hypertension even in this age period

In the older child approaching adolescence or in the very acutely ill younger child it may be necessary to increase the dosage to 80 mg daily to obtain a favorable response Usually a maximal dose of 60 mg daily is given in the first week of illness and is reduced to 40 mg for the remainder of the period of treatment

Frequently a normal platelet level is obtained with three weeks of steroid treatment in which case the drug is tapered off during the fourth week In the majority of patients only one course of treatment is required Regardless of recovery blood counts are carried out at regular intervals for several months and years to assure the permanency of the remission Maintenance therapy with smaller doses (2 5 to 15 mg per day) has been advocated when the platelets have reached normal levels[1] [0] in the hope that this will assure a permanent remission and reduce the need for later splenectomy This plan may be followed in children with a history of relapsing purpura in whom splenectomy seems inevitable In the few patients in whom this form of treatment has been carried out the minimal dosage necessary to maintain adequate platelet response was 15 mg There are several objections to maintenance therapy in children as a routine procedure persistent and excessive hypercorticism generalized osteoporosis and retardation of growth If the response with steroids in successive courses is transient or inadequate better results are occasionally obtained by the intramuscular administration of three or four single daily doses of 60 units of corticotrophin in a gelatin menstruum In an occasional patient after several courses of treatment with corticosteroids the platelet count may become stabilized at levels of approximately 100 000 per cubic millimeter without maintenance therapy and without evidence of purpura Under these conditions splenectomy may be postponed

Splenectomy Splenectomy was introduced as a form of treatment in this disease by Kaznelson [1] Splenectomy is indicated if with adequate therapy thrombocytopenia persists for six months or longer especially when associated with recurrent purpura or overt bleeding provided secondary or symptomatic forms of thrombocytopenic purpura have been eliminated and megakaryocytes are present in normal or increased numbers in the bone marrow The finding of a few or no megakaryocytes warrants a search for an underlying condition and is a contraindication to splenectomy An expectant policy is followed beyond the six month period if the bleeding tendency is not manifested despite thrombocy

topenia and the patient is asymptomatic In such children a decision is usually reached when at the end of a year platelet levels either return to normal or are eventually associated with hemorrhagic manifestations Complete clinical and hematologic remissions can be expected in about 85 per cent of children with the chronic disease in whom splenectomy is performed In a series comprising *patients of all ages splenectomy was successful in 81 per cent of those with* idiopathic thrombocytopenic purpura whereas corticosteroid therapy was successful in only 38 per cent [1]

When splenectomy is contemplated preoperative preparation with steroids is useful in controlling hemostasis during the procedure [4] The therapeutic administration of the steroids is of equal or even greater importance in those patients who have had intermittent rather than continuous treatment In the unprepared patient adrenal insufficiency may compromise the patient s ability to *deal with acute stress which may develop during splenectomy Hydrocortisone* is preferable to prednisone or prednisone like compounds in the preoperative and operative periods During childhood 50 mg of hydrocortisone is given intramuscularly at twelve hour intervals on the day preceding operation and the blood pressure is observed One dose is repeated in the morning of the operation and another is given during the operation by slow intravenous drip It is a good plan to continue treatment for several days following the operation Because of the excessive increase in the number of platelets in many of these patients *postoperatively and because of the danger of thrombosis platelet levels should* be followed routinely Anticoagulant therapy is advised if platelet counts before the end of the first week postoperatively persist at levels over 1 million per cubic millimeter [44] The occasional susceptibility of the splenectomized child to overwhelming infection has prompted the routine use of a broad spectrum antibiotic for at least two years following the operation in our clinic [6]

It should be pointed out that in certain children especially those in the preadolescent and adolescent groups splenectomy may not provide the anticipated cure We have encountered *several children in whom thrombocytopenia with o ert bleeding* accompanied by a positive Coombs test with or without hemolytic anemia recurred following a remission of several months to a year The failure of platelets to increase following splenectomy may indicate the presen e of a *more widespread disease and a fatal outcome A case in point is* that of a boy whose spleen was removed at 14 years of age for thrombocytopenic purpura The platelet count following the operation remained depressed and could only be restored to normal levels by large doses of steroids After a remis ion of nine months hemolyti anemia occurred in which the Coombs test was negative Soon after recovery from the anemia marked thrombocytopenia recurred w h ch could not be controlled and the patient eventually succumbed with a fatal hemorrhage The Coombs test was positive in the final episode despite the absence of an anemia due to a hemolytic process Throughout the entire course the test for l pus erythematosus was consistently negative Such a combination of circ mstances is suggestive of the concept of Evans and co workers [8-9] who emphasized the immunologic *relationship between acquired hemolytic anemia and thrombocytopenic purpura*

Congenital Thrombocytopenic Purpura

Congenital thrombocytopenic purpura occurs infrequently but the literature contains many case reports and studies bearing on its pathogenesis [6] [64] [3] It appears *in infants born of mothers with idiopathic thrombocytopenic purpura*

who either have had a splenectomy or are symptomless and unaware of a lowered content of platelets of normal mothers or of mothers with drug induced thrombocytopenic purpura such as follows the ingestion of quinine

Etiology Current concepts implicate an immunoallergic mechanism in the causation of the disease in the infant [?] According to Harrington [4] one third of the patients do not have any demonstrable autoantibodies for platelets and are assumed to have a defect of platelet formation from megakaryocytes as the sole factor causing thrombocytopenia Mothers with this type of idiopathic thrombocytopenia give birth to normal infants On the other hand patients with autoimmune idiopathic thrombocytopenic purpura possess a circulating antibody which damages both platelets and megakaryocytes Mothers with this variety of thrombocytopenia give birth to infants with purpura The antibodies which cross the placenta into the fetal circulation consist of autoagglutinins as well as isoagglutinins for the platelets of the mother and infant

In the case of normal mothers it has been postulated that the mother develops isoagglutinins for the baby s platelets presumably on the basis of platelet incompatibility between mother and infant in a manner analogous to Rh sensitization in infants with erythroblastosis She may also have been sensitized to platelet antigen in a previous transfusion or to fetal platelets of a different antigenic composition during pregnancy The precise nature of the immune substance is not always obvious

Quinine induced thrombocytopenic purpura in a mother and her newborn infant has been observed[61] with in vitro evidence of quinine platelet antibodies in the plasma of the mother and of the infant just after delivery The administration of quinine before delivery resulted in thrombocytopenia and purpura due to the presence of preformed antibody from maternal sensitization to the drug originally given in childhood

Prognosis There have been several families in which consecutive babies have had neonatal thrombocytopenic purpura The prognosis in the infant is usually good with the restoration of a normal number of platelets within the first two or three weeks of life and usually not beyond three months Frequently the baby appears normal at birth but develops purpura several hours after delivery The course may not be benign especially when the pregnancy is associated with overt purpura In one report consecutive infants born of a mother who had been splenectomized for idiopathic thrombocytopenic purpura succumbed on the first day of life [1C] Megakaryocytes are increased in patients with primary thrombocytopenic purpura and when markedly decreased or absent signify the presence of an underlying condition In patients with congenital thrombocytopenic purpura however megakaryocytes may be normal in number or frequently depressed The persistence of an amegakaryocytic condition beyond the first months of life carries a poor prognosis

Treatment In the seriously ill infant ACTH is administered intramuscularly in the first two days in a dosage of 25 mg daily in four divided doses and for the next two days with half the amount For the remainder of the week prednisone is substituted It is given orally in a dosage of 10 to 20 mg daily in four divided doses The dosage is reduced to half the amount in the second week and tapered off gradually in the third week The maintenance of ACTH and steroid

therapy beyond these periods depend upon the severity of the disease As in patients with idiopathic thrombocytopenic purpura individual adjustment of dosage is necessary Although the mode of action in unknown hemorrhage is usually controlled by these agents regardless of platelet response With severe bleeding or purpura transfusions of fresh blood are advisable

Thrombocytopenia Induced by Drugs

Thrombocytopenia induced by drugs may be either amegakaryocytic or megakaryocytic [90] In the former group are the myelosuppressive chemical and physical agents such as x ray radiation which involves the other bone marrow elements as well as the platelets to produce hypoplastic and aplastic anemia A variety of drugs have been incriminated as being among those producing thrombocytopenia These include among others Mesantoin thyroid depressing drugs sulfonamides benzol arsenic and chloramphenicol Here the effect on the bone marrow is due either to individual idiosyncrasy or to overdosage and emphasizes the need for routine blood studies as a check on toxicity Drugs used currently in the treatment of patient s with leukemia Hodgkin s disease and other disorders of the lymphoma group are capable of producing thrombocytopenia by megakaryocytic aplasia.

Megakaryocytic thrombocytopenia may result from sensitization produced in the patient by previous administration of the drug as in the case of Sedormid quinine and quinidine It has been shown that the addition of Sedormid to platelets suspended in the serum of a sensitized patient causes the agglutination without complement and lysis with it Patients who have recovered from purpura caused by Sedormid possess an antiplatelet antibody in the circulating blood which is capable of destroying platelets The fact that normal serum had no such effect indicates that the abnormality in the blood of such a person lies in the serum and not in the platelets

In patients with thrombocytopenic purpura due to Sedormid and probably other drugs the antibody acts on megakaryocytes platelets and capillary endothelium In those with nonthrombocytopenic purpura the platelets for some unknown reason escape injury The drug presumably combines with vascular endothelium rendered antigenic and therefore capable of uniting with antibody Thrombocytopenia due to quinine presents a similar mechanism—namely an antibody combined with antigen consisting of the drug and platelets [85]

Aplastic Anemia With Onset as Congenital Thrombocytopenic Purpura

A newborn infant was recently observed who had what appeared to be congenital thrombocytopenic purpura After the first weeks of life however anemia and subsequently granulocytopenia became associated with the depletion of platelets Although a normal bone marrow was present at the end of the first month by the seventh month the blood bone marrow and clinical findings were consistent with aplastic anemia Although this sequence so early in life is exceptional it is significant nevertheless that congenital thrombocytopenia usually benign may represent the initial stage of aplastic anemia

Chronic Hypoplastic Thrombocytopenia With Depression of Megakaryocytes

Chronic hypoplastic thrombocytopenia with depression of megakaryocytes is occasionally observed in pediatric practice in patients without congenital anomalies and consists of persistent thrombocytopenia with a depletion of mega karyocytes and periodic attacks of epistaxis purpura or both There is no involvement of red or white cell elements nor any coagulation defect These patients must be observed for the possible ultimate development of the total picture of aplastic anemia or for evidence of secondary thrombocytopenia due to causes such as infection drugs and leukemia or other infiltrative disorders

For treatment of this condition successive monthly courses of prednisone or substitutes are administered especially if bleeding or purpura is excessive If there is difficulty in maintaining platelet levels to prevent bleeding maintenance therapy may be attempted on a dosage of 2 5 to 15 mg daily Continuous therapy even with minimal dosage necessitates alertness for the development of hyperadrenocorticism When the administration of steroids has proved ineffective in patients with severe and recurrent nosebleeds large doses of ascorbic acid (1 000 mg daily) have occasionally resulted in improvement The value of removing the spleen is debatable especially since this organ is not enlarged

Congenital Hypoplastic Thrombocytopenia (Primary Amegakaryocytic Thrombocytopenia)

Rarely there appears to be a congenital absence or a marked reduction in the number of megakaryocytes without a reduction in the other elements of the bone marrow often coexisting with multiple deformities [8] Among the first of these cases to be described was that reported by Greenwald and Sherman[43] in which defective formation of megakaryocytes was associated with anomalies of the heart and thymus gland

In another patient first observed at 3 months of age thrombocytopenic purpura was associated with congenital dislocation of the hip and bilateral absence of the radii [90] After mild improvement with ACTH and cortisone therapy splenectomy was performed at 10 months of age Bleeding stopped the number of platelets increased and one year later the platelets numbered 350 000 per cubic millimeter In a similar case[8] of primary aplasia of the megakaryocytes and absence of both radii splenectomy resulted in a transitory increase in the number of platelets Improvement was not maintained and the infant succumbed with cerebral hemorrhage Similar congenital abnormalities with bleeding manifestations from birth based on amegakaryocytic bone marrow may present evidence of a leukemoid reaction

The majority of patients succumb in the first weeks and months of life despite transfusions and splenectomy From a broad point of view these cases of congenital hypoplastic thrombocytopenia with skeletal and cardiac anomalies may be classified as a neonatal equivalent of the Fanconi syndrome Clinical and hematologic features do not ordinarily appear in patients with the Fanconi syndrome until later childhood

Thrombotic (Thrombohemolytic) Thrombocytopenic Purpura

Thrombotic thrombocytopenic purpura is an acute febrile disease characterized by thrombocytopenic purpura severe hemolytic anemia and transitory focal neurologic signs and symptoms The varied clinical features are due to widespread intracapillary and intra arteriolar thrombi affecting most frequently the brain kidneys heart and spleen The occlusions are due not to agglutinated platelets alone but also to the vascular wall involvement with conspicuous proliferation The possibility of thrombotic thrombocytopenic purpura should be considered whenever an unusually severe or fulminating purpura associated with thrombopenia is encountered [10]

Pathogenesis It has been postulated that thrombotic thrombocytopenic purpura represents an immunohematologic disorder in which the autoimmune process is set in motion against the red cell platelets megakaryocyte and vessel wall [3] The association with marked eosinophilia in the occasional patient[13] tends further to support this concept The hypersensitive basis of the histologic picture is analogous to other collagen diseases particularly periarteritis nodosa and lupus erythematosus and to the necrotizing vascular lesion produced in experimental animals [1] by a variety of agents such as foreign proteins sulfonamides and iodine

Clinical Course and Laboratory Findings The course is usually rapidly progressive terminating fatally within a few days to several weeks from the onset In a few patients the illness is more prolonged and of a relapsing nature Petechiae and ecchymoses are few or numerous enlargement of the spleen and liver occurs in many of the patients The blood shows the evidence of a hemolytic anemia with jaundice in one half of the patients Anemia reticulocytosis microspherocytosis leukocytosis thrombocytopenia and erythroid hyperplasia of the bone marrow are present The Coombs test is ordinarily negative occasionally positive [99]

Treatment Splenectomy ACTH cortisone and antibiotics are of transitory value Blood and platelet transfusions are supportive only Anticoagulants such as heparin are without effect since the occlusions are caused by a primary pathologic reaction of the vascular wall to a damaging agent and in the absence of fibrin in the thrombi [86]

Eczema, Thrombocytopenia, and Anemia (Aldrich's Syndrome)

The combination of chronic eczema complicated by thrombocytopenic purpura and chronic suppurative otitis media has been the subject of several reports [4, 44, 110] Bloody diarrhea recurrent infection anemia epistaxis and a fatal outcome are additional features Persistent thrombocytopenia despite normal numbers and morphology of megakaryocytes has been noted [7] The pattern is that of a sex linked recessive disease with transmission by unaffected females to male members

Milk allergy was strongly suspected as a major factor in producing the eczema in one series [42] An impressive feature common to the children in the latter group was the rapidity with which they developed fulminating infections after splenectomy [4] The outcome is fatal despite ACTH and steroid therapy Although there

is a clinical resemblance to Letterer-Siwe disease, the genetic pattern and combination of symptoms differentiate this condition from reticuloendotheliosis and other conditions in which either eczema or purpura is present. No clear evidence is available demonstrating a deficiency of an immune response to most antigens. Normal or slightly elevated gamma globulin levels were noted in one series.

Thrombocytopenia Following Transfusions

A tendency to bleed based on platelet depletion has been described in patients receiving large amounts of compatible banked blood.[6] Several reasons have been cited for thrombocytopenia resulting from transfusions. When bleeding is excessive, the patient loses both platelets and substances required in the coagulation process. In blood collected by ordinary blood bank procedures, the agglutination and destruction of platelets are considerable, so that the donor contributes inadequate amounts of viable platelets during transfusion. No significant thrombocytopenia results in patients receiving small amounts of blood over a period of days or weeks.

Another factor contributing to thrombocytopenia is the possible depressive effect of transfusions on platelet formation, as is known to occur with erythropoiesis. A factor which reduces platelets has also been described in normal plasma which, acting alone or with the recipient's diluted platelets, operates to produce thrombocytopenia.[89] A combination of these factors has been shown to reduce the number of platelets in infants with erythroblastosis receiving replacement transfusions, although overt bleeding is rare.[6]

Hemolytic Anemia, Thrombocytopenic Purpura, and Renal Disease (Hemolytic Uremic Syndrome)

Hemolytic uremic syndrome[18] consists of hemolytic anemia, thrombocytopenia and renal disease clinically resembling acute glomerulonephritis with hematuria and proteinuria. The red cells in the hemolytic episodes are characteristically distorted, irregularly contracted and fragmented, but they return to normal during remissions.[8] Spherocytes have also been noted in small numbers. Splenectomy and cortisone have been without value. The only effective treatment is blood transfusion, which may have to be repeated at frequent intervals because of the rapid destruction of transfused cells. Thrombocytopenia necessitates the use of fresh blood.[8] This syndrome is separated from autoimmune hemolytic disease by the negative Coombs test and the unusual red cell morphology. In many aspects it resembles a form of hypersensitivity and has been regarded as related to thrombotic thrombocytopenic purpura. This illness may be rapidly fatal[36] or be marked by recurrences,[8] and the patient may recover spontaneously after a single episode. In the group of patients who recover spontaneously, renal damage did not persist.

Cyclic Thrombocytopenic Purpura Related to the Menstrual Cycle

A physiologic decrease in the number of platelets occurs in the two weeks before menstruation in the majority of women, reaching the lowest levels during

the first day of menstruation Easy bruising is a reflection of this alteration A gradual increase in the number of platelets takes place during the following two weeks [9] Intermittent thrombocytopenic purpura has been described which was confined to the menstrual period and was followed by a spontaneous remission [43] In patients with idiopathic thrombocytopenic purpura the platelet count may be lowest during menstruation and highest during ovulation [4] Severe menorrhagia may be an indication of this change and sometimes the sole clinical manifestation of this disease

Hemangioma and Thrombopenia

Thrombocytopenic purpura has been reported in association with hemangioma in infancy [1] A hemorrhagic tendency and bleeding into the hemangiomatous site may occur The lesions may be so extensive as to result in a fatal outcome as thrombopenia develops [9] The thrombopenia hemangioma syndrome shows an increased number of young and agranular megakaryocytes in the bone marrow The occurrence of platelet thromboses within the vessels of the hemangioma conceivably results in their sequestration in the tumor with peripheral depletion of platelets [11] Elimination of the hemangioma results in return of the platelet count to normal whereas splenectomy is without effect on the blood dyscrasia [9] [41] Multiple hemangiomas and thrombocytopenia have also been reported Surgical excision resulted in severe bleeding [38]

NONTHROMBOCYTOPENIC PURPURAS

Allergic Purpura (Anaphylactoid Purpura Henoch Schönlein Purpura)

Allergic purpura refers to a type of nonthrombocytopenic purpura accompanied by a pleomorphic urticaria like and purpuric type of cutaneous eruption in combination with gastrointestinal symptoms (Henoch s purpura) painful swelling of the joints (Schonlein s purpura rheumatica) and a tendency toward renal involvement These features are grouped together as the Henoch Schonlein syndrome with the realization that these symptom complexes can occur individually in combination or in sequence

Etiology and Pathogenesis The relationship of the purpuric state to allergy is suggested by the symptoms of urticaria diffuse erythema and subcutaneous and submucous extravasations of blood and lymph Although hypersensitivity is generally considered to underlie the Henoch Schonlein syndrome a definite allergen is only rarely identified Food and less frequently bacterial infection and drugs including antibiotics are suspected as the exciting factors A large variety of foods have been chiefly implicated—eggs milk chocolate wheat nuts and beans and to a lesser extent fish pork, lamb chicken and a variety of fruits In one patient an insect bite led to joint swelling blood tinged stools and hematuria [11] Acute vascular purpura has also been reported following immunization with Asiatic influenza vaccine Although a relationship is suggested clinically skin tests are generally negative and more is gained by eliminating the suspected foods from the diet and observing for the subsidence of symptoms

is a clinical resemblance to Letterer-Siwe disease, the genetic pattern and combination of symptoms differentiate this condition from reticuloendotheliosis and other conditions in which either eczema or purpura is present. No clear evidence is available demonstrating a deficiency of an immune response to most antigens. Normal or slightly elevated gamma globulin levels were noted in one series.

Thrombocytopenia Following Transfusions

A tendency to bleed based on platelet depletion has been described in patients receiving large amounts of compatible banked blood.[8] Several reasons have been cited for thrombocytopenia resulting from transfusions. When bleeding is excessive, the patient loses both platelets and substances required in the coagulation process. In blood collected by ordinary blood bank procedures, the agglutination and destruction of platelets are considerable, so that the donor contributes inadequate amounts of viable platelets during transfusion. No significant thrombocytopenia results in patients receiving small amounts of blood over a period of days or weeks.

Another factor contributing to thrombocytopenia is the possible depressive effect of transfusions on platelet formation as is known to occur with erythropoiesis. A factor which reduces platelets has also been described in normal plasma which, acting alone or with the recipient's diluted platelets, operates to produce thrombocytopenia.[59] A combination of these factors has been shown to reduce the number of platelets in infants with erythroblastosis receiving replacement transfusions, although overt bleeding is rare.[8]

Hemolytic Anemia, Thrombocytopenic Purpura, and Renal Disease (Hemolytic Uremic Syndrome)

Hemolytic uremic syndrome[16] consists of hemolytic anemia, thrombocytopenia and renal disease clinically resembling acute glomerulonephritis with hematuria and proteinuria. The red cells in the hemolytic episodes are characteristically distorted, irregularly contracted and fragmented, but they return to normal during remissions.[6] Spherocytes have also been noted in small numbers. Splenectomy and cortisone have been without value. The only effective treatment is blood transfusion which may have to be repeated at frequent intervals because of the rapid destruction of transfused cells. Thrombocytopenia necessitates the use of fresh blood. This syndrome is separated from autoimmune hemolytic disease by the negative Coombs test and the unusual red cell morphology. In many aspects it resembles a form of "hypersensitivity" and has been regarded as related to thrombotic thrombocytopenic purpura. This illness may be rapidly fatal[36] or be marked by recurrences,[8] and the patient may recover spontaneously after a single episode. In the group of patients who recover spontaneously, renal damage did not persist.

Cyclic Thrombocytopenic Purpura Related to the Menstrual Cycle

A physiologic decrease in the number of platelets occurs in the two weeks before menstruation in the majority of women, reaching the lowest levels during

An infectious agent (a drug or toxin for example) may supposedly form a complex with capillary endothelium or platelets along the lines suggested by Ackroyd with Sedormid therapy thus initiating active immunization As further confirmation of the concept of Henoch Schonlein disease as a hyperimmune dis ease was the application of a precipitin test using a preparation of the aorta of newborn infants A positive precipitin test with decalcified serum was obtained in six out of eight patients with allergic purpura and three out of four patients with periarteritis nodosa [91]

Clinical Features Henoch Schonlein purpura occurs more commonly in males than in females is slightly more frequent in children and adolescents and has a peak incidence in children from 4 to 15 years of age The average duration of the disease is about four weeks The disease is marked by a tendency toward recurrences In a series of 131 pediatric patients with allergic purpura Allen and associates[4] reported an age range of 6 months to 16 years with a median of 4 years Three fourths of these patients were under 7 years of age and about one third were either 2 or 3 years old Within six weeks of the onset of the disease 40 per cent of these patients had one or more recurrences after a period of well being Recurrences were more common in patients over 2 years of age than in younger patients The occurrence of the disease in several siblings in one family is rare but it has been reported [9] The disease is ushered in by headaches anorexia and abdominal pain Fever is rarely over 101 F The onset is variable and early in the course symptoms from the skin gastrointestinal tract or joints or any combination may predominate Localized edema occurs on the backs of the hands and around the eyes face scalp and lips Subconjunctival hemorrhage may be present Although skin lesions usually occur initially colicky abdominal pain and gastrointestinal bleeding or joint symptoms may precede any type of skin rash for prolonged periods Younger patients tend to show more frequent edema of the soft tissue of the scalp face eyes hands and feet and older children are more prone to gastrointestinal and renal manifestations [4]

Skin The specific skin lesion progresses from recurrent crops of urticarial lesions to maculopapules which are at first pink and then red Petechial hemorrhages occur in a zone of erythema with new lesions appearing every few days Ecchymoses urticarial wheals and edema are common The lesions tend to spread forming larger patches often with a hemorrhagic component as a later development The most commonly affected sites are the buttocks lower back extensor surfaces of the elbows and arms and the back of the lower leg ankle and foot Rash on the calves and thighs has been prominent in some patients A petechial and purpuric eruption simulating the embolic phenomena of meningococcal sepsis may occur Epistaxis and bleeding from the gums are rare Histologic changes are restricted to the small vessels of the corium which are surrounded by an acute inflammatory exudate [3] Tissue eosinophilia suggests a local hypersensitive reaction

Abdominal Symptoms Abdominal pain frequently referred to the umbilical region is common Recurrent colicky pain is the most striking symptom of the disease The attacks are associated with vomiting often with melena consisting of the passage of blood and mucus The lower ileum is usually involved

The high incidence of preceding upper respiratory infections often with beta hemolytic *streptococci* and the similarity between the latent period of one to three weeks until the appearance of the *hemorrhagic* manifestations have suggested both a close association between *Henoch* Schonlein purpura and acute nephritis and the hyperimmune nature of both *diseases* On the other hand Bywaters and co workers[1] found in a series of sixty four cases that although Henoch Schonlein purpura was *preceded by* an upper respiratory infection in 72 per cent of their *patients group A* beta hemolytic streptococci were isolated in only one fourth of the *patients investigated* This evidence was intermediate between that found in patients with rheumatic fever and in a control series of children admitted for nonrheumatic carditis Thus there is no clear evidence that this *type of specific* infection is an invariable causative factor It may well be that the increasing use of the *sulfonamide* drugs and antibiotics in connection with the treatment of acute upper respiratory infections may be responsible for the apparent *increasing* incidence of the Henoch Schonlein syndrome[39]

A disturbance of the vascular endothelium resulting in increased capillary fragility and permeability is a basic factor in the symptomatology Osler[69] was among the first to call attention to the association between arthritis *and intestinal* symptoms and the erythema group of skin diseases and to emphasize the renal *complication* The erythematous exanthem of the Henoch Schonlein syndrome *has been* differentiated from true purpura in which the surrounding skin is normal[2] However the two conditions are not always separable and true *purpura* in the sense of this definition is often observed in children in conjunction with *other features* of Henoch Schonlein purpura

The *Henoch* Schonlein syndrome has been linked[3,3] with acute nephritis *rheumatic fever and* polyarteritis nodosa and other collagen diseases They have overlapping clinical characteristics and a similar pathogenesis based on an antigen antibody reaction involving the endothelium of blood vessels Henoch *Schonlein* purpura can be classified as an immunovascular disorder resulting in a *generalized blood* vessel disturbance an acute perivasculitis or angiitis affecting the skin joints intestinal tract and renal glomeruli[17] Kreidberg and associates *postulated* a latent period between the remission of a preceding infection and the appearance of purpura during which an immunologic mechanism develops The *resulting* antigen antibody reaction at the vascular level would explain the generalized vascular damage The histopathologic features of acute necrotic involvement of vessel walls and hemorrhage which are prime features have led to the designation of this disease as allergic angiitis[109] The pathologic lesion in skin and kidney biopsies consists of fibrinoid thrombi in capillaries and an associated endothelial and perivascular reaction[98]

An antiserum made experimentally from vascular endothelium has been found to produce diffuse hemorrhagic purpura in the skin and internal organs Following the direction of previous Japanese investigators Clark and Jacobs using suspensions of dog vascular endothelium from the aorta and vena cava produced a rabbit antiserum which when injected in dogs produced a generalized nonthrombocytopenic hemorrhagic purpura The resulting lesions corresponded to the diffuse vasculitis involving small blood vessels with perivascular collections of polymorphonuclear cells lymphocytes and macrophages observed in Henoch Schonlein disease changes which provide a basis for the increased permeability of small blood vessels

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although both the stomach and jejunum may be affected Colic when severe may suggest intestinal obstruction intussusception and acute appendicitis These symptoms may precede or accompany the purpuric rash Perforation is a rare occurrence

Laparotomy is occasionally undertaken to eliminate these possibilities although at operation no remedial surgery is usually required Exception is taken in the case of the patient with intussusception a rare complication of Henoch Schonlein purpura in which operation is urgently indicated[94] The abdominal symptoms are related to edema or extravasations of blood or serosanguineous fluid into the wall of the intestine Despite the fact that the alimentary lesions have been compared to the skin purpura they have also been interpreted as local vasospasms secondary to damage to the arterioles With atypical abdominal symptoms surgical intervention should not be undertaken without questioning as to previous attacks of purpura The tip of the spleen is occasionally palpable

Joint Involvement Joint symptoms vary from transient puffiness of a single joint to recurrent warm and painful swelling of many joints often associated with a limp The knees and ankles are most commonly affected The swelling is due to periarticular involvement rather than intra articular bleeding or effusion There is no permanent damage to the joints

Renal Complications Renal involvement has been estimated to occur in as high as one half of the children with Henoch Schonlein purpura[6,7] and constitutes by far the most serious complication of the disease Glomerulonephritis is manifested by gross and microscopic hematuria cylindruria and proteinuria Although the majority of children make a complete recovery some may develop a latent chronic nephritis and eventual renal failure[4,10] The prognosis must be guarded in those with prolonged hematuria

The pathology of the kidneys in allergic purpura has been subject to close analysis and varied interpretation Renal biopsy has made it possible to correlate the clinical and laboratory findings with the actual pathologic status of the kidney[5b,6,98a] It is realized that the severity and chronicity of the glomerulonephritis vary from case to case in this disease According to Vernier and associates[98a] the acute glomerular lesion is characterized by segmental glomerular proliferation and occlusion of capillaries by Schiff positive fibrinoid material with organized hyaline material or segmental scars in the older lesions These authors regard the lesion not as similar to acute glomerular nephritis but rather as part of a diffuse vascular disease with lesions resembling those seen in patients with disseminated lupus erythematosus Bergstrand and associates[5b] interpret the renal lesions as remnants of an inflammatory process similar to glomerulonephritis with the histologic changes favoring a good prognosis even in patients with persistent hematuria

Neurologic Complications Serious neurologic complications are rare In two children the exanthema intestinal colic vomiting arthralgia and nephritis were accompanied by subarachnoid hemorrhage which was fatal in one patient[7] In another patient swelling of an ankle joint preceded the purpuric rash and was followed by colic and fatal cerebral hemorrhage

Laboratory Findings The bleeding time coagulation studies and clot retraction are normal Platelets are normal in number Anemia and leukocytosis occur only in patients with severe blood loss and eosinophilia is rare The tourniquet test is positive in only 25 per cent of patients

Diagnosis The typical purpuric erythematous eruption colic and articular and periarticular swelling in a patient with a normal platelet count differentiates the Henoch Schonlein syndrome from the thrombocytopenic purpuras The normal coagulation studies serve further to separate this condition from established bleeding disorders

Course and Prognosis The disease is variable in its manifestations and duration In the majority of patients there is only a single acute episode which clears up spontaneously in a month Recurrences are common In its mildest form the disease runs its course in a few days with transient purpura on the extremities and lower trunk and fleeting abdominal or joint pain or both

At the other extreme are the serious and fatal forms often with an insidious onset with progressive purpura severe arthralgia gastrointestinal hemorrhages abdominal pain hypertension and kidney and cerebral hemorrhage In patients with recurrent abdominal pain there is constant concern over the need for surgical intervention

A chronic course with exacerbations over a period of years is not uncommon In some patients successive seasonal attacks often occur in the spring with complete remissions in the intervening months Rarely the disease progresses with the eventual clinical picture of rheumatoid arthritis and transient purpura The prognosis for the outcome of a single attack is good and ultimate recovery even with repeated attacks is generally good

Chronic renal disease constitutes the most serious aspect and the persistence of proteinuria and red cells in the urine necessitates close supervision Prognosis is especially guarded when there is nervous system involvement or when abdominal symptoms are sufficiently urgent to require surgical exploration

Treatment The treatment is essentially symptomatic Bed rest is required during an attack In cases which appear to be related to upper respiratory infections either acute or chronic in nature foci of infection should be investigated Only when pathogenic organisms are definitely isolated should antibacterial therapy be instituted Antibiotics may be of value although infrequently in our experience when given they should be used with discrimination and caution to avoid provoking or aggravating already existing purpura Occasionally in patients with Henoch Schonlein purpura associated with hemolytic streptococci tonsillectomy has been effective in preventing recurrences

Antihistamine drugs vitamins C and K rutin and hesperidin have been of questionable value The most favorable results have been obtained by the detection of an offending food and its elimination from the diet Suspected foods should therefore be systematically investigated for their possible role in etiology although this is usually not too successful Chocolate nuts fish tomatoes and other foods mentioned previously should be investigated for their effect on recurrence of attacks of purpura

ACTH and the adrenocortical steroids are commonly employed but with only

fair results It might be expected that the allergic type of *hypersensitivity as* exemplified by Henoch Schonlein purpura should respond because of the beneficial effect of the steroids in other members of the collagen group of diseases to which it is related Although these drugs do not prevent relapses *nor modify* the course of nephritis they are worthy of trial In patient with moderately *severe* allergic purpura steroids (prednisone or substitutes) are given orally *according* to age 30 to 60 mg daily in divided doses for brief periods of time *Thus treat*ment should be supervised especially for the effect on blood *pressure elevation* and aggravation of abdominal signs In patients with severe and *uncontrollable* disease ACTH or hydrocortisone is given intravenously by *drip over an eight* hour period in a dosage of 25 mg and 100 mg respectively ACTH is given intramuscularly when oral administration of steroids has been *ineffective*

In the series of Allen and associates[4] corticosteroids *were of no value in the management of the skin manifestations* or renal involvement They were found to be useful *in arthralgia* soft tissue swelling and scalp edema These authors emphasize *the need for corticosteroids* in sufficient dosage for the relief of abdominal *pain* The *steroids* did *not* however alter the frequency of recurrences nor the duration of illness

As has already been *mentioned* clinical signs indicating abdominal involvement *usually do not* necessitate surgical intervention An exception is made for the *patient with severe* colic suggesting intussusception The latter has been *ascribed to intramural* hematomas of the intestinal tract serving as a leading *point for peristaltic invagination*[94] Reduction or resection of the gangrenous segment is usually successful

Congenital Vascular Defects

Pseudohemophilia Pseudohemophilia known as the von Willebrand syndrome has been described in connection with coagulation disorders (see Chapter 26) It was shown that this syndrome could be classified into two distinct groups both possessing a capillary defect with prolonged bleeding time One group was demonstrated to be associated with marked deficiency in antihemophilic globulin (pseudohemophilia B) in the other the abnormality was confined to the vascular defect (pseudohemophilia A) with no abnormality of coagulation

Hereditary Hemorrhagic Telangiectasia (Rendu-Osler Weber Disease) Rendu Osler Weber disease is a hereditary condition characterized by the *presence of* telangiectases either localized or generalized and by a tendency to bleed *from* these lesions It is transmitted as a simple dominant affecting both sexes although a generation may be skipped The individual lesions are slightly raised are 1 to 4 mm in size have a bright red and violaceous appearance and consist of localized dilatations of capillaries and venules in the skin and mucous membrane Because of their exposed nature they give rise to profuse hemorrhage either spontaneously or as a result of trauma The onset is typically in childhood with epistaxis due to a localized lesion in the nasal mucous membrane The telangiectases increase in number and become widespread in adult life[83]

Recurrent and severe epistaxis in childhood and in older persons justifies nasopharyngoscopic examination for possible demonstration of telangiectases The

lesions are located most commonly in the nasal mucous membrane and less often on the palate tongue conjunctivae lips scalp ears and the face and under the fingernails Visceral lesions account for gastrointestinal[105] genitourinary and pulmonary bleeding and less often for bleeding in other organs In patients with pseudohemophilia in whom nosebleed is also severe the bleeding time is prolonged in contrast to normal findings in patients with hereditary telangiectases Borderline cases occur[104]

Therapy consists of protection against local trauma and topical and hemostatic agents including thermocautery transfusions and iron for severe hypochromic anemia when the loss of blood is severe The disease is rarely fatal since patients rarely die from hemorrhage

Ehlers Danlos Syndrome The Ehlers Danlos syndrome is due to a constitutional and congenital dysplasia of the mesenchyme and comprises the following clinical features friability of the skin (dermatorrhexia) and of the blood vessels overextensibility of the joints (arthrochalasis) hyperelasticity of the skin pseudotumors and freely movable subcutaneous nodules[61 96] These pea sized nodules consist of subcutaneous fat With the onset of walking injuries sustained by the infant result in gaping incised wounds with bleeding due to tearing of the adjacent blood vessels The pathologic background is represented by scanty subcutaneous tissue thinning of collagenous fibers and an increase of the elastic tissue in the corium which is arranged in irregular and coarse bundles With the reduced subcutaneous tissue and friability of the skin the blood vessels are susceptible to trauma There is no bleeding tendency Bleeding and coagulation time are normal There is no epistaxis oozing from mucous surfaces nor undue bleeding from wounds or tooth extraction This syndrome is regarded as a hereditary disease with dominant transmission occurring in both sexes but not in all patients is there a familial incidence[61]



Fig 47 Ehlers Danlos syndrome Hyperlaxity of the joints is especially prominent in the fingers (From Smith C H Dermatorrhexis [Ehlers-Danlos Syndrome] J Pediat 14 632, 1939)



Fig 48



Fig 49

Fig 48 Loose and hyperelastic skin stretched beyond normal limits returns to normal position when released (From Smith C H Dermatorrhexis [Ehlers Danlos Syndrome] J Pediat 14 632 1939)

Fig 49 High power photomicrograph of skin (×260) Note that the malpighian layer of the epidermis is reduced in cellular content The dense reticulum of coarse elastic elements in the corium and the diminution of collagen fibers are important factors in the production of the gaping incised wounds characteristic of the syndrome (From Smith C H Dermatorrhexis [Ehlers Danlos Syndrome] J Pediat 14 632 1939)

There is no specific treatment that can alter the fundamental abnormalities of this condition Therapy consists in the attempt to increase the deposition of fat in the subcutaneous layer to act as a buffer against trauma

Purpura Fulminans

Purpura fulminans is a rare form of nonthrombocytopenic purpura characterized by anemia fever shock and sudden and rapidly spreading skin hemorrhages usually occurring symmetrically in the lower extremities[86] Marked prostration occurs almost exclusively in young children and follows an infectious disease The course is usually rapidly fatal within a few days Of the diseases of childhood purpura fulminans follows scarlet fever most often[13, 101] but it may also be associated with varicella[86] and measles Food has also been incriminated as a causative agent The basic lesion consists of extensive vasculitis which leads to necrosis of tissues Because it resembles a hypersensitivity reaction it is often

regarded as a variant of Henoch Schonlein purpura although the cardinal features of the latter (mucous membrane bleeding and joint intestinal and renal symptoms) are absent Necrotizing vasculitis may be so extensive as to require amputation of the lower legs Contributing to the clinical picture of purpura fulminans is the diminution in clotting factors such as labile factor (factor V)[3] and fibrinogen[18] Rarely the platelet count is decreased[16]

Treatment ACTH the adrenocortical steroids intravenous fluids and antibiotics should be given Transfusions of fresh blood and plasma are especially useful when a clotting factor is absent

Waterhouse Friderichsen Syndrome

Waterhouse Friderichsen syndrome is a rapidly fatal form of severe purpura associated with adrenal hemorrhage during the course of meningococcemia or other severe septicemias Fever shock prostration vascular collapse and widespread hemorrhages are prominent features The disease usually affects infants from 2 to 15 months of age and in 90 per cent of all cases it occurs in children under 9 years old ACTH and adrenocortical steroids are used in treatment and have occasionally been helpful[63]

Idiopathic Pulmonary Hemosiderosis

Idiopathic pulmonary hemosiderosis is a disease occurring most frequently in childhood and in young adults and is characterized by dyspnea cyanosis fever cough blood streaked sputum and pallor with laboratory evidence of iron deficiency anemia These features are associated with the basic pathologic process in which blood is extravasated in the pulmonary alveoli by repeated hemorrhages

Clinical Course The diagnosis of idiopathic pulmonary hemosiderosis should be considered in patients with severe cough dyspnea dullness rales bronchial breathing and anemia The course is one of remissions and exacerbations with intermittent hemoptysis and hematemesis Acute episodes last two to ten days but occasionally persist for several weeks[10, 4, 84, 85, 106, 111] Mild jaundice appears after a few days Occasionally the disease occurs with a rapidly fatal outcome without recurrent manifestations[84] Physical examination during an acute attack may not be striking The roentgenogram of the chest shows a variety of findings diffuse homogeneous opacities bilateral perihilar patchy infiltration or diffuse pulmonary infiltrations with increase in bronchial markings extending from the hilar region Clearing of the pulmonary fields occurs subsequently but roentgenographic examination shows a residual flecked reticular pattern or a persistent miliary type of infiltration radiating from the hilus At the onset the disease resembles virus pneumonia or a severe upper respiratory infection The most frequent peaks of incidence are at 1 to 2 years of age and at 13 to 15 years of age[8] An apical systolic murmur may be present the spleen may be palpable and in children in whom the disease has become chronic the liver is enlarged The disease must be differentiated from pulmonary hemosiderosis secondary to mitral stenosis Readmission to the hospital is sought for respiratory distress fever and hemoptysis

Sputum gastric washings, and aspiration from lung puncture[37] reveal macrophages laden with granules of hemosiderin stained deep blue with the Prussian blue reaction Intra alveolar pulmonary blood loss accounts for a hypochromic microcytic iron deficiency anemia with reticulocytosis In two children aged 3 and 8 years under observation evidence of the iron deficiency anemia was further confirmed by a low serum iron content of the blood and a moderate increase in

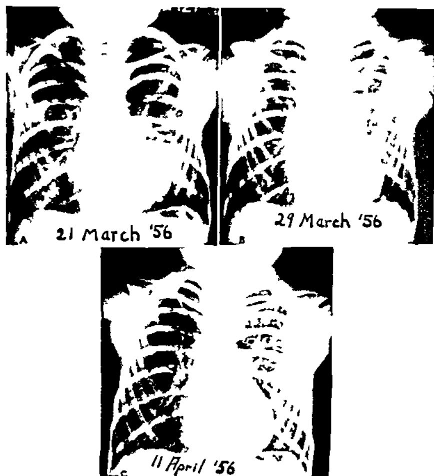


Fig 50 Roentgenograms of a 4 year-old girl with pulmonary hemosiderosis Note perihilar and pulmonary infiltration due to extravasation of blood into the alveoli by repeated hemorrhages *A* and *B* The attacks are recurrent with substantial clearing during convalescence *C*

latent iron binding capacity Studies of the anemia and iron distribution in patients with idiopathic pulmonary hemosiderosis[6] revealed that during the acute phase there was an excessive plasma iron turnover and an iron kinetic pattern characteristic of an iron deficiency anemia and hemorrhage and not of hemolysis The tuberculin and Coombs tests are negative the platelet and coagulation factors are normal The transient jaundice and elevation of indirect reacting bilirubin in the serum following acute or subacute episodes indicate the degradation of intra alveolar blood

Prognosis After a course of several years the disease usually ends with an acute fatal exacerbation Terminal cardiorespiratory failure results from the effects of recurrent pulmonary bleeding In a review by Soergel[87] the average duration was 2 9 years the shortest 5 weeks and the longest 10 years Of thirty two patients nine had died ten were still active five showed no progression of the disease and eight had become symptom free

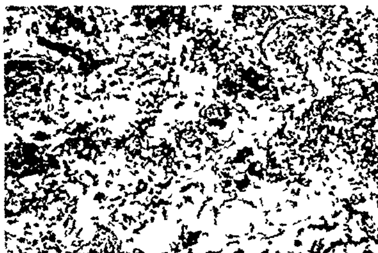


Fig 51 –Pulmonary hemosiderosis Fibrous thickening of the alveolar septa of the lung showing broken thin and sparse strands of elastic tissue (×150) Extensive hemorrhage into alveolar spaces bronchioles and small bronchi is evidenced by the presence of diffuse hemosiderosis (Heavily stained areas are indicative of iron deposition)

Pathology The basic pathologic process is repeated intra alveolar hemorrhages massive in patients with acute attacks and of variable amounts in those with subacute or chronic phases There is no hemosiderosis of other organs The pulmonary findings in a 4 year old child with a fourteen month history of several exacerbations typify the usual fatal case There was a diffuse fibrous thickening of the alveolar septa which showed broken thin and sparse strands of elastic tissue clumps of golden pigment laden macrophages in numerous alveolar spaces and extensive hemorrhages into alveolar spaces bronchioles and small bronchi When a special stain is employed the pigment in the macrophages stains blue indicating the presence of iron or evidence of old hemorrhage Hyaline membrane formation was also found in many alveoli and in some bronchioles

Pathogenesis The etiology of this disease has been ascribed to a primary developmental defect of the elastic fibers with their fragmentation resulting in stasis within capillary vessels and hemorrhages The failure to find destruction of elastic tissue in all patients led Steiner[93] to regard essential pulmonary hemosiderosis as an antigen antibody reaction caused by a still unknown sensitizing agent inducing the production of autoantibodies With the lungs as a shock organ the antigen antibody reaction produces capillary dilatation stasis diapedesis rhexis and increased destruction of red corpuscles and deposits of hemosiderin

Treatment For the effects of acute and chronic blood loss into the lungs almost continuous iron therapy transfusions bed rest and oxygen are required Iron from the extravasated blood in the pulmonary alveoli is available for hemoglobin formation following each episode[31] Antibiotics are employed for superimposed infection Based on these immunoallergic hypotheses steroids and ACTH have been advocated Favorable reports with this treatment require further evaluation because of the great variations in the frequency and severity of attacks Similarly splenectomy designed to reduce the severity of the bleeding episodes needs to be subjected to more prolonged trial

Miscellaneous Purpuric Disorders (Nonthrombocytopenic)

Vilberg and Brown[98] reviewed a number of syndromes consisting of capillary disorders with and without coagulation defects Purpura simplex refers to mild skin purpura unassociated with specific blood disorders Hereditary familial purpura simplex is a condition in which spontaneous cutaneous purpura occurs on a transmissible basis involving several generations [1] The bleeding time is normal the tourniquet test is positive This type of purpura occurring in association with congenital ptosis of the eyelids has been described in eleven members of a family representing four generations[3]

REFERENCES

1 Ackroyd J F Three Cases of Thrombocytopenic Purpura Occurring After Rubella With a Review of Purpura Associated With Infections Quart J Med 18 299 1949
2 Ackroyd J F Allergic Purpura Including Purpura Due to Foods Drug and Infections Am J Med 14 605 1953
3 Adelson E Heitzman E J and Fennessey J F Thrombohemolytic Thrombocytopenic Purpura A M A Arch Int Med 94 42 1954
4 Aldrich R A Steinberg A G and Campbell D C Pedigree Demonstrating a Sex Linked Recessive Condition Characterized by Draining Ears Eczematoid Dermatitis and Bloody Diarrhea Pediatrics 13 133 1954
4a Allen D M Diamond L K and Howell D A Anaphylactoid Purpura in Children (Schonlein Henoch Syndrome) Review With a Follow Up of the Renal Complications A M A J Dis Child 99 833 1960
5 Allison A C Acute Haemolytic Anemia With Distortion and Fragmentation of Erythrocytes in Children Brit J Haemat 3 1 1957
5a Angle R M and Alt H L Thrombocytopenic Purpura Complicating Infectious Mononucleosis Report of a Case and Serial Platelet Counts During the Course of Infectious Mononucleosis Blood 5 449 1950
6 Apt L Pollycove M and Ross J F Idiopathic Pulmonary Hemosiderosis Study of Anemia and Iron Distribution Using Radio Iron and Radiochromium J Clin Invest 36 1150 1957
7 Balf C L The Alimentary Lesion in Anaphylactoid Purpura Arch Dis Childhood 26 20 1951
7a Basu R Perforation of the Bowel in Henoch Schonlein Purpura Arch Dis Childhood 34 342 1959
8 Bell A D Mold J W Oliver R A M and Shaw S Study of Transfused Platelets in a Case of Congenital Hypoplastic Thrombocytopenia Brit M J 2 629 1956
8a Berglund C and Broberger O Treatment of Idiopathic Thrombocytopenic Purpura in Childhood Acta paediat (Suppl 117) 48 7 1959
8b Bergstrand A Bergstrand C C and Bucht H Kidney Lesions Associated With Anaphylactoid Purpura in Children Acta paediat 49 57 1960

8c Bergstrand C G and Bucht H Renal Biopsy in Children Acta paediat (suppl 117) 48 1-6 1959
9 Bogin M and Thurmond J Hemangioma With Purpura Thrombocytopenia and Erythrocytopenia A M A Am J Dis Child 81 675 1951
10 Browning J R and Houghton J D Idiopathic Pulmonary Hemosiderosis Am J Med 20 374 1956
11 Burke D M and Jellinek H L Nearly Fatal Case of Schoenlein Henoch Syndrome Following Insect Bite A M A Am J Dis Child 88 772 1954
12 Bywaters E G L Isdale I and Kempton J J Schonlein Henoch Purpura Evidence for a Group A Beta Haemolytic Streptococcal Aetiology Quart J Med 26:161 1957
12a Carpenter A G Wintrobe M M Fuller L A Haut A and Cartwright C E Treatment of Idiopathic Thrombocytopenic Purpura J A M A 171:1911 1959
13 Chambers W N Holyoke J B and Wilson R F Purpura Fulminans Report of Two Cases Following Scarlet Fever New England J Med 247:933 1952
14 Clark W G and Jacobs E Experimental Non thrombocytopenic Vascular Purpura a Review of the Japanese Literature With Preliminary Confirmatory Report Blood 5 320 1950
15 Clement D H and Diamond L K Purpura in Infants and Children A M A Am J Dis Child 85 259 1953
16 Crawford S E and Riddler J G Purpura Fulminans A M A J Dis Child 97:198 1959
17 Dameshek W Acute Vascular Purpura, an Immuno-vascular Disorder Blood 8:382 1953
17a Dameshek W Controversy in Idiopathic Thrombocytopenic Purpura J A M A 173 1025 1960
18 Dameshek W and Miller E B The Megakaryocytes in Idiopathic Thrombocytopenic Purpura, a Form of Hypersplenism Blood 1 27 1946
19 Dameshek W and Reeves W H Exacerbation of Lupus Erythematosus Following Splenectomy in Idiopathic Thrombocytopenic Purpura and Autoimmune Hemolytic Anemia, Am J Med. 21 560 1956
20 Dameshek W Rubio F Jr Mahoney J P and Reeves W H Treatment of Idiopathic Thrombocytopenic Purpura (ITP) With Prednisone J A M A 166 1805 1958
20a Dargeon, H W Adiao A C and Pack G T Hemangioma and Thrombocytopenia J Pediat. 54 285 1959
21 Davis E Hereditary Familial Purpura Simplex Review of 27 Families Lancet 1 145 1941
22 Davis E The Schonlein-Henoch Syndrome of Vascular Purpura Blood 3 129 1948
23 Derham R J and Rogerson M M The Schonlein-Henoch Syndrome and Collagen Disease Arch Dis Childhood 27 139 1952
24 Derham R J and Rogerson M M The Schonlein-Henoch Syndrome With Particular Reference to Renal Sequelae Arch Dis Childhood 31 364 1956
25 Diggs L W and Hewlett J S A Study of the Bone Marrow From Thirty Six Patients With Idiopathic Hemorrhagic (Thrombopenic) Purpura Blood 3 1090 1948
26 Doan, C A Hypersplenism Bull New York Acad Med 25 625 1949
27 Emery J L Gordon R R Rendle Short J Varadi S and Warrack A J M Congenital Amegakaryocytic Thrombocytopenia With Congenital Deformities and a Leukemoid Blood Picture in the Newborn. Blood 12 567 1957
28 Evans R S and Duane R T Acquired Hemolytic Anemia the Relation of Erythrocyte Antibody Production to Activity of the Disease the Significance of Thrombocytopenia and Leukopenia Blood 4 1196 1949
29 Evans R S, Takahashi K Duane A B Payne P and Liu C Primary Thrombocytopenic Purpura and Acquired Hemolytic Anemia Evidence for a Common Etiology A.M.A. Arch Int. Med. 87 48 1951
30 Faloon W W Greene R W and Lozner E L Hemostatic Defect in Thrombocytopenia as Studied by Use of ACTH and Cortisone Am J Med 13 12, 1952

Treatment For the effects of acute and chronic blood loss into the lungs almost continuous iron therapy transfusions bed rest and oxygen are required Iron from the extravasated blood in the pulmonary alveoli is available for hemoglobin formation following each episode [31] Antibiotics are employed for superimposed infection Based on these immunoallergic hypotheses steroids and ACTH have been advocated Favorable reports with this treatment require further evaluation because of the great variations in the frequency and severity of attacks Similarly splenectomy designed to reduce the severity of the bleeding episodes needs to be subjected to more prolonged trial

Miscellaneous Purpuric Disorders (Nonthrombocytopenic)

Valberg and Brown[98] reviewed a number of syndromes consisting of capillary disorders with and without coagulation defects Purpura simplex refers to mild skin purpura unassociated with specific blood disorders Hereditary familial purpura simplex is a condition in which spontaneous cutaneous purpura occurs on a transmissable basis involving several generations [1] The bleeding time is normal the tourniquet test is positive This type of purpura occurring in association with congenital ptosis of the eyelids has been described in eleven members of a family representing four generations [3]

REFERENCES

1 Ackroyd J F Three Cases of Thrombocytopenic Purpura Occurring After Rubella With a Review of Purpura Associated With Infections Quart J Med 18 299 1949
2 Ackroyd J F Allergic Purpura Including Purpura Due to Foods Drugs and Infections Am J Med 14 605 1953
3 Adelson E Heitzman E J and Fennessey J F Thrombohemolytic Thrombocytopenic Purpura A M A Arch Int Med 94 42 1954
4 Aldrich R A Steinberg A G and Campbell D C Pedigree Demonstrating a Sex Linked Recessive Condition Characterized by Draining Ears Eczematoid Dermatitis and Bloody Diarrhea Pediatrics 13 133 1954
4a Allen D M Diamond L K and Howell D A Anaphylactoid Purpura in Children (Schonlein Henoch Syndrome) Review With a Follow Up of the Renal Complications A M A J Dis Child 99 833 1960
5 Allison A C Acute Haemolytic Anemia With Distortion and Fragmentation of Erythrocytes in Children Brit J Haemat 3 1 1957
5a Angle R M and Alt H L Thrombocytopenic Purpura Complicating Infectious Mononucleosis Report of a Case and Serial Platelet Counts During the Course of Infectious Mononucleosis Blood 5 449 1950
6 Apt L Pollycove M and Ross J F Idiopathic Pulmonary Hemosiderosis Study of Anemia and Iron Distribution Using Radio Iron and Radiochromium J Clin Invest 36 1150 1957
7 Balf C L The Alimentary Lesion in Anaphylactoid Purpura Arch Dis Childhood 26 20 1951
7a Basu R Perforation of the Bowel in Henoch Schonlein Purpura Arch Dis Childhood 34 342 1959
8 Bell A D Mold J W Oliver R A M and Shaw S Study of Transfused Platelets in a Case of Congenital Hypoplastic Thrombocytopenia Brit M J 2 629 1956
8a Berglund G and Broberger O Treatment of Idiopathic Thrombocytopenic Purpura in Childhood Acta paediat (suppl 117) 48 7 1959
8b Bergstrand A Bergstrand C G and Bucht H Kidney Lesion Associated With Anaphylactoid Purpura in Children Acta paediat 49 57 1960

52 Kolars C P and Spink W W Thrombopenic Purpura as a Complication of Mumps J A M A 168 2213 1958

53 Koller F Gasser C Krusi G and Muralt G de Purpura fulminans nach Scharlach mit Factor V Mangel und antithrombin Uberschuss Acta haemat 4 33 1950

54 Komrower G M and Watson G H Prognosis in Idiopathic Thrombocytopenic Purpura of Childhood Arch Dis Childhood 29 502 1954

55 Kreidberg M B Dameshek W and Latorraca R Acute Vascular (Schonlein Henoch) Purpura an Immunologic Disorder? New England J Med 253 1014 1955

56 Krevans J R and Jackson D P Hemorrhagic Disorder Following Massive Whole Blood Transfusions J A M A 159 171 1955

57 Krivit W and Good R A Aldrich s Syndrome (Thrombocytopenia Eczema and Infection in Infants) Studies of the Defense Mechanisms A M A J Dis Child 97 137 1959

58 Landolt, R F Kongenitale (neonatale) Thrombopenien Helvet paediat acta 3 3 1948

59 Lewis I C and Philpott M G Neurological Complications in the Schonlein Henoch Syndrome Arch Dis Childhood 31 369 1956

60 MacFarlane R G The Mechanism of Haemostasis Quart J Med 10 1 1941

61 Mauer A M DeVaux L O and Lahey M E Neonatal and Maternal Thrombocytopenic Purpura Due to Quinine Pediatrics 19 84 1957

62 McAllenney P F and Kristan J J Thrombopenic Purpura in the Newborn Am J Dis Child 78 401 1949

63 Minot G R Purpura Hemorrhagica With Lymphocytosis Acute Type and Intermittent Menstrual Type Am J M Sc 192 445 1936

64 Morris M B Thrombocytopenic Purpura in the Newborn Arch Dis Childhood 29 75 1954

65 Nelson J and Goldstein N Nature of Waterhouse Friderichsen Syndrome Report of Case With Successful Treatment With Cortisone J A M A 146 1193 1951

66 Newton W A Jr and Zuelzer W W Idiopathic Thrombopenic Purpura in Childhood New England J Med 245 879 1951

67 Oliver T K and Barnett H L The Incidence and Prognosis of Nephritis Associated With Anaphylactoid (Schoenlein Henoch) Purpura in Children A M A Am J Dis Child 90 544 1955

68 Osler W On the Visceral Manifestations of the Erythema Group of Skin Diseases Am J M Sc 127 1 1904

69 Pepper H Liebowitz D and Lindsay S Cyclical Thrombocytopenic Purpura Related to the Menstrual Cycle A M A Arch Path 61 1 1956

70 Pohle F J Blood Platelet Count in Relation to Menstrual Cycle in Normal Women Am J M Sc 197 40 1939

70a Rabinowitz Y and Dameshek W Systemic Lupus Erythematosus After Idiopathic" Thrombocytopenic Purpura Review Ann Int. Med 52 1 1960

71 Rich A R Hypersensitivity in Diseases Harvey Lect 42 106 1946-1947

72 Robson H N and Walker C H M Congenital and Neonatal Thrombocytopenic Purpura Arch Dis Childhood 26 175 1951

73 Schoen E J King A L and Duane R T Neonatal Thrombocytopenic Purpura Pediatrics 17 72 1956

74 Schulman I Pierce M Lukens A Currimbhoy Z and Fort E A Factor in Normal Human Plasma Which Stimulates Platelet Production Chronic Thrombocytopenic Purpura Due to Its Deficiency A M A J Dis Child 98 633 1959

74a Schulman I Pierce M Lukens A and Currimbhoy Z Studies on Thrombopoiesis I A Factor in Normal Human Plasma Required for Platelet Production Chronic Thrombocytopenia Due to Its Deficiency Blood 16 943 1960

75 Schwartz, S O Prognostic Value of Marrow Eosinophils in Thrombocytopenic Purpura Am J M Sc 209 579 1945

30a Ferguson A W Rubella As a Cause of Thrombocytopenic Purpura Pediatrics 25 400 1960
31 Finch S C and Finch C A Idiopathic Hemochromatosis an Iron Storage Disease Iron Metabolism in Hemochromatosis Medicine 34 381 1955
32 Fisher B Zuckerman G H and Douglass R C Combined Inheritance of Purpura Simplex and Ptosis in Four Generations of One Family Blood 9 1199 1954
33 Fisher E R and Creed D L Thrombotic Thrombocytopenic Purpura Report of a Case With Discussion of Its Tinctorial Features Am J Clin Path 25 620 1955
34 Fisher O D and Kraszewski T M Thrombocytopenic Purpura Following Measles Arch Dis Childhood 27:144 1952
35 Gairdner D The Schonlein Henoch Syndrome (Anaphylactoid Purpura) Quart J Med 17 95 1948
36 Gasser C Gautier E Steck A Siebenmann R E and Oechslin E *Hamolytisch uramische Syndrome* bilaterale Nierenrindennekrosen bei akuten erworbenen hamolytischen Anamien Schweiz med Wchnschr 85 905 1955
37 Gellis S S Reinhold J D L and Green S Use of Aspiration Lung Puncture in the Diagnosis of Idiopathic Pulmonary Hemosiderosis A M A Am J Dis Child 85 303 1953
38 Gilon E Ramot B and Sheba C Multiple Hemangiomata Associated With Thrombocytopenia Remarks on the Pathogenesis of the Thrombocytopenia in This Syndrome Blood 14 74 1959
39 Glaser J Letter to Editor Pediatrics 19 1152 1957
40 Goldburgh H L and Couley B A Postpubertal Menorrhagia and Its Possible Relations to Thrombocytopenic Purpura Hemorrhagica Am J M Sc 200 499 1940
41 Good T A Carnazzo S F and Good R A Thrombocytopenia and Giant Hemangioma in Infants A M A Am J Dis Child 90 260 1955
42 Greene R W Faloon W W and Lozner L L The Use of ACTH in Preparing Patients With Idiopathic Thrombocytopenic Purpura for Splenectomy Am J M Sc 226 203 1953
43 Greenwald H M and Sherman I Congenital Essential Thrombocytopenia Am J Dis Child 38 1245 1929
44 Gross R E The Surgery of Infancy and Childhood Its Principles and Techniques Philadelphia 1953 W B Saunders Co
45 Halvorsen S Cortisone Treatment of Idiopathic Pulmonary Hemosiderosis Acta paediat 45 139 1956
46 Harrington W J The Clinical Significance of Antibodies for Platelets Sang 25 712 1954
47 Harrington W J Sprague C C Minnich V Moore C V Ahlvin R C and Dubach R Immunologic Mechanisms in Idiopathic and Neonatal Thrombocytopenic Purpura Ann Int Med 38 433 1953
48 *Heal F C and Kent C Purpura Fulminans With Afibrinogenemia Canad M A J* 69 367 1953
49 Huntley C C and Dees S C Eczema Associated With Thrombocytopenic Purpura and Purulent Otitis Media Pediatrics 19 351 1957
50 Irvin J M and Snowden P W Idiopathic Pulmonary Hemosiderosis A M A J Dis Child 93 182 1957
50a Kaplan E Congenital and Neonatal Thrombocytopenic Purpura J Pediat 54 644 1959
51 Kasabach H H and Merritt K K Capillary Hemangioma With Extensive Purpura Report of a Case Am J Dis Child 59 1063 1940
51a Kaznelson P Verschwinden der Hamorrhagischen Diathese bei einen Falle von essentieller Thrombopenie (Frank) nach Milzexstirpation Wien klin Wchnschr 29 145 1916
51b Kelemen E Cserhati I and Tanos B Demonstration and Some Properties of Human Thrombopoietin in Thrombocythaemic Sera Acta haemat 20 350 1958

98b Vernier R L Farquhar M G Brunson J C and Good, R A Chronic Renal Disease in Children Correlation of Clinical Findings With Morphologic Characteristics Seen by Light and Electron Microscopy A M A J Dis Child 96 306 1958
99 Villasenor J B and Ambrosius K Thrombotic Thrombocytopenic Purpura Report of a Case With Some Unusual Characteristics Ann Int Med 46 378 1957
100 Watson Williams E J Macpherson, A I S and Davidson S The Treatment of Idiopathic Thrombocytopenic Purpura a Review of Ninety Three Cases Lancet 2 221 1958
101 Webb B D Dubs E J and Conrad E Postscarlatinal Gangrene With Prolonged Prothrombin Time J Pediat 30 76 1947
102 Wedgwood R J P and Klaus M H Anaphylactoid Purpura (Schonlein Henoch Syndrome) A Long Term Follow up Study With Special Reference to Renal Involvement Pediatrics 16 196 1955
103 Welch R G Thrombocytopenic Purpura and Chicken pox, Arch Dis Childhood 31 38 1956
104 Wells E B *Hereditary Hemorrhagic Telangiectasia Am J M Sc 211 577 1946*
105 Whitney L H and Barritt A S Jr Spontaneous and Hereditary Thrombopenic Purpura in a Mother and Two Sons Am J Dis Child 64 705 1942
106 Wigod, M Idiopathic Pulmonary Hemosiderosis Report of a Case in Early Childhood With Severe Anemia New England J Med 253 413 1955
107 Wile S A and Sturgeon P Thrombotic Thrombocytopenic Purpura Review of the Subject With a Report of Three Cases in Children Pediatrics 17 882 1956
108 Williams G A and Brick I B Gastrointestinal Bleeding in Hereditary Hemorrhagic Telangiectasia, A M A Arch Int Med 95 41 1955
109 Winkelman R K Clinical and Pathologic Findings in the Skin in Anaphylactoid Purpura (Allergic Angiitis) Proc Staff Meet Mayo Clin 33 277 1958
110 Wolff J A and Pertucio M A Sex Linked Genetic Syndrome in a Negro Family Manifested by Thrombocytopenia, Eczema Bloody Diarrhea Recurrent Infection Anemia and Epistaxis A M A J Dis Child 93 74 1957
111 Wyllie W G Sheldon W Bodian M and Barlow A *Idiopathic Pulmonary Haemosiderosis* Quart J Med 17 25 1948

76 Schwartz S O and Kaplan S R Thrombocytopenic Purpura the Prognostic and Therapeutic Value of the Eosinophil Index an Analysis of 100 Cases Am J M Sc 219 528 1950

77 Shaw S and Oliver R A M Congenital Hypoplastic Thrombocytopenia With Skeletal Deformities Blood 14 374 1959

78 Shumway C N Jr and Miller G An Unusual Syndrome of Hemolytic Anemia Thrombocytopenic Purpura and Renal Disease Blood 12 1045 1957

79 Silver H K Aggeler P M and Crane J T Hemangioma (Capillary and Cavernous) With Thrombocytopenic Purpura Report of a Case With Observations at Autopsy Am J Dis Child 76 513 1948

80 Singer K Thrombotic Thrombocytopenic Purpura In Snapper I and Dock William (editors) Advances in Internal Medicine vol 6 Chicago 1954 Year Book Publishers Inc p 195

81 Smith C H Dermatorrhexis (Ehlers Danlos Syndrome) J Pediat 14 632 1939

82 Smith C H Erlandson M Schulman I and Stern G Hazard of Severe Infections in Splenectomized Infants and Children Am J Med 22 390 1957

83 Smith J I and Lineback M I Hereditary Hemorrhagic Telangiectasia Nine Cases in One Negro Family With Special Reference to Hepatic Lesions Am J Med 17 41 1954

84 Smith W E and Fienberg R Early Nonrecurrent Idiopathic Pulmonary Hemosiderosis in an Adult Report of a Case New England J Med 259 808 1958

85 Soergel K H Idiopathic Pulmonary Hemosiderosis Review and Report of Two Cases Pediatrics 19 1101 1957

86 Stamey C C Mauley J H and London A H Purpura Fulminans Following Chicken pox North Carolina M J 17 115 1956

87 Steen E and Torp K H Encephalitis and Thrombocytopenic Purpura After Rubella Arch Dis Childhood 31 470 1956

88 Stefanini M Management of Thrombocytopenic States A M A Arch Int Med 95 543 1955

89 Stefanini M and Chatterjea J B Studies on Platelets IV Proc Soc Exper Biol & Med 79 623 1952

90 Stefanini M and Dameshek W The Hemorrhagic Disorders a Clinical and Therapeutic Approach New York 1955 Grune & Stratton Inc

91 Stefanini M and Mednicoff I B Therapeutic Effect of Pituitary Adrenocorticotrophic Hormone in a Case of Henoch Schonlein Vascular Purpura J A M A 144 1372 1950

92 Stefanini M Piomelli S Mele R Ostroski J T and Colpoys W P Acute Vascular Purpura Following Immunization With Asiatic Influenza Vaccine New England J Med 259 9 1958

93 Steiner B Essential Pulmonary Haemosiderosis as an Immuno haematological Problem Arch Dis Childhood 29 391 1954

94 Steinhardt I D and Jonas A F Coexistence of Intussusception and Henoch's Purpura New England J Med 257 553 1957

95 Steinkamp R Moore C V and Doubeck W G Thrombocytopenic Purpura Caused by Hypersensitivity to Quinine J Lab & Clin M d 45 18 1955

96 Summer G K The Ehlers Danlos Syndrome A M A J Dis Child 91 419 1956

97 Tidy H L Haemorrhagic Diathesis Angiostaxis Lancet 2 365 1926

97a Towner C H Familial Incidence of the Henoch Schonlein Syndrome Brit M J 2 1385 1959

98 Valberg L S and Brown C M Haemorrhagic Capillary Disorder Associated With Antihaemophilic Globulin Deficiency Medicine 37 181 1958

98a Vernier R L Kidney Biopsy in the Study of Renal Disease In Pediat Clin North America Symposium on Recent Clinical Advances (Kelley V C editor) Philadelphia 1960 W B Saunders Co vol 7 p 353

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